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ABSTRACTS

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CONTENTS

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* 3760 volunteer abstracts, 21 symposium/workshop abstracts.

CHRONOLOGICAL LIST OF SESSIONS

(See page v for Thematic List of Sessions.)

Continued in Part Two

THEMATIC LIST OF SESSIONS*

Theme A: Development and Plasticity

Session Number	Title	Type	Day	Time
83.	Axon Outgrowth, Growth Cones, and Guidance Mechanisms I	Poster	Tue	8:30 AM
266.	Axon Outgrowth, Growth Cones, and Guidance Mechanisms II	Slide	Fri	8:30 AM
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.

34.	The Assembly of Topographic Maps in the Central Nervous System: A Cartographer's Delight	Symp.	Mon	1:00 PM
137.	Genetic Correlates of Mental Disease	Symp.	Wed	8:30 AM
3.	Regulation of Acetylcholine Receptor and Channel Properties During Development	Symp.	Mon	8:30 AM
172.	Sex Hormones and Neural Development: Implications for the Genesis of Sexual Differentiation	Symp.	Wed	1:00 PM

Theme B: Cell Biology

Session Number	Title	Type	Day	Time
224.	Axonal Organelles, Transport, and Specific Staining	Poster	Thu	8:30 AM
237.	Axoplasmic Transport	Slide	Thu	1:00 PM
243.	Blood–Brain Barrier	Poster	Thu	1:00 PM
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
102.	Drug Effects on Receptors	Poster	Tue	8:30 AM
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
103.	Membrane Biophysics I	Poster	Tue	8:30 AM
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
134.	Presynaptic Mechanisms: Peripheral Nervous System	Poster	Tue	1:00 PM
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
285.	Brain Metabolism	Poster	Fri	8:30 AM
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

2 SYMPOSIUM. FEDERAL SUPPORT FOR THE NEUROSCIENCES, CONCEPTION TO FUNDING: THE PROCESS AND PROGRAMS. S.H. Koslow, National Institute of Mental Health (NIMH)(Chairman); Donald Woodward*, Office of Naval Research; George W. Irving, III*, Air Force Office of Scientific Research/NL; Zaven Khachaturian, National Institute on Aging (NIA); Norman A. Krasnegor*, National Institute of Child Health and Human Development (NICHD); Constance W. Atwell*, National Eye Institute (NEI); Eugene Streicher*, National Institute of Neurological and Communicative Disorders and Stroke (NINCDS); James Brown, National Science Foundation (NSF).

This symposium will present an overview of the numerous factors which enter into the decision making processes of Governmental support for basic and applied Neuroscience research. Major support for this research is provided through the NSF, NINCDS, NIMH, NEI, NIA, NICHD, as well as from the different services of the Department of Defense. A description of each of these funding sources will be presented, along with specific identification of programs, as well as points of contact. An indepth description of each agency will follow outlining the "missions" of each, as well as presenting the overall areas of interest and responsibility. Major emphasis will be placed on the funding mechanisms utilized by investigators to apply for grant support. In addition, other support mechanisms will also be described, e.g., training grants, fellowships, small grants, cooperative agreements and contracts. For each of the funding agencies the process of applying for grants or contracts (including pre-submission contact with program directors), will be described. This will provide details of the processes of assignment to funding organization and review group, the process of review, constitution of review groups, second level of review, and decisions of funding by agency staff.

Following these presentations the full panel of Federal program directors will hold an open discussion session.

3 SYMPOSIUM. REGULATION OF ACETYLCHOLINE RECEPTOR AND CHANNEL PROPERTIES DURING DEVELOPMENT. Y. Kidokoro, The Salk Institute (Chairman); J. Patrick, The Salk Institute; Y. Nakajima, Purdue University; B. Sakmann , Max-Plank Institut; G.D. Fischbach, Washington University.

Multidisciplinary approaches are rapidly expanding our appreciation of the function and regulation of the acetylcholine (ACh) receptor. In this symposium we would like to bring together several different systems and techniques to present an overview of developmental changes in ACh receptors.

The genes that code for the ACh receptor from Torpedo have been cloned. cDNA clones have been isolated for two of the subunits (α and γ). These clones are being used to determine the structure and organization of the ACh receptor genes. They have been largely sequenced and the complete amino acid sequence of the ACh receptor is being deduced. These clones are also useful as probes to study the expression of the ACh receptor genes. The biosynthesis of ACh receptor will be discussed in relation to the finding that cDNA for the γ subunit codes for a precursor peptide containing a 'signal' sequence (Patrick, J.). Functional ACh receptors appear on the muscle surface membrane before synaptic transmission starts. One type of dispersed intramembrane particles seen in freeze-fracture electron micrographs correlate well with the appearance of functional ACh receptors assayed by ACh iontophoresis (Nakajima, Y.). During formation of the neuromuscular junction ACh receptors undergo various changes. Nerve induces high ACh receptor density along the contact soon after initial functional transmission. Properties of individual ACh receptor channels also change. The mean open time of ACh receptor channels shortens at the subjunctional membrane in the neonatal rat. The shortening is due to shift of ACh receptor channel population with a long mean open time to a short one. Since the turnover time for ACh receptors in the subsynaptic membrane is already long at this time, this shift of population is likely the result of modification of existing ACh receptor channels (Sakmann, B.). On the contrary the mean open time does not undergo developmental changes in the chick, although the density of ACh receptors increases at the subjunctional region. The clustering of receptors by itself, therefore, is not responsible for the open time shortening. During this period the rate of receptor degradation stays comparable to value obtained at extrajunctional sites on denervated adult muscles (Fischbach, G.). In Xenopus nerve-muscle cultures neurons from embryonic neural tube mediate changes in both the density and the functional properties of ACh receptors in the muscle cell. The neuronally mediated increase in the receptor density is restricted to the region closely associated to the nerve-muscle contact. In contrast to this regional effect of nerve, a nerve-dependent alteration in functional properties is observed for junctional and non-junctional receptor alike (Kidokoro, Y.).

4 WORKSHOP. THEORETICAL APPROACHES TO MOTOR CONTROL. E. Bizzi, M.I.T. (Chairman); R. Llinas, N. Y. Univ. Med. Ctr. (Chairman); L. M. Optican*, NEI, National Institutes of Health; A. Pellionisz, N. Y. Univ. Med. Ctr.; T. Poggio*, M.I.T.

There is little doubt that the problem of understanding the properties and the operations of the biological motor controller is an exceedingly difficult task. Experimentation in this area cannot proceed without theories which reflect and deal with the constraints imposed by physics and by the computational complexities related to the problems of coordinate transformation, kinematics and dynamics. Presently, in the field of motor control, there is a spectrum of theoretical approaches ranging from "System Theories" to Tensorial models of the brain, to control strategies based upon the mechanical properties of muscles. These ideas will be discussed and contrasted with computational theories derived from the field of Artificial Intelligence. The speakers participating in this symposium will describe these approaches, their relationship to experimental findings, and their possible impact on future experimentation.

The powerful electrophysiological techniques that are now used in many laboratories have certainly provided us with a great wealth of data on movement. We feel, however, that the predominant, almost extreme, empiricism which characterizes research in motor control might be in the long run disadvantageous for progress in this field. Our proposal is then directed at presenting and discussing those theories that might have a chance to guide experimentation in the areas of motor and sensory control.

Finally, an overview of current theoretical approaches to the problem of motor control may offer an interesting paradigm for other areas of neuroscience.

***Indicates nonmember of the Society for Neuroscience.**
PO indicates abstracts that are published only.

5.1 EFFECTS OF STRABISMUS ON RESPONSIVITY, SPATIAL RESOLUTION, AND CONTRAST SENSITIVITY OF CAT LATERAL GENICULATE NEURONS. Kim R. Jones, Ronald E. Kalil, and Peter D. Spear. Depts. of Psychology and Ophthalmology, Univ. of Wisconsin, Madison, WI 53706.

Previous studies have reported that rearing cats with esotropia causes two major physiological effects in the lateral geniculate nucleus (LGN): (1) a loss of responsive cells in the portion of lamina A1 that receives inputs from the peripheral (>10°) temporal retina of the deviated eye, and (2) a loss of spatial resolution among X-cells that have central (<5°) receptive fields. We extended these findings by quantifying the responsiveness of peripheral A1 cells, and by obtaining spatial contrast sensitivity functions for central X-cells.

Recordings were made from the portion of lamina A1 representing the peripheral retina in 3 normal and 3 esotropic cats. In normals, an average of 3.3 cells/penetration was encountered, and all but 1 of 43 cells were responsive. Mean peak response to an optimal stimulus was 177 spikes/sec. In esotropes, 2.7 cells/penetration were encountered, and all 30 cells studied were responsive. Mean peak response was 174 spikes/sec. The differences between normals and esotropes in these and other measures of responsiveness were not statistically significant. There also was no difference in percentage of X- or Y-cells between the two groups. Thus, esotropia had no effect on the responsivity of LGN cells with inputs from the peripheral temporal retina.

Spatial resolution was measured for X-cells with central receptive fields in 6 normal, 5 esotropic, and 4 exotropic cats. Stimuli were drifting (2 Hz) sine wave gratings of 30 cd/m^2 mean luminance and 64% contrast. In normals, mean spatial resolution for 43 X-cells was 3.1 c/°. Rearing with esotropia significantly reduced X-cell spatial resolution; the mean for 38 X-cells was 2.1 c/°. This reduction was seen consistently among individual cats. In 2 cats raised with one eye converged and the other lid-sutured, X-cell spatial resolution was intermediate between normal and esotropic cats. This suggests that the spatial resolution loss in esotropes is at least partly due to abnormal binocular interactions. In contrast to esotropia, rearing with exotropia had no significant effect on X-cell spatial resolution; the mean for 43 X-cells was 3.0 c/°. However, there was more variability among cats in this group than in the others.

Other measures of spatial contrast sensitivity were normal in esotropic cats. These included maximum sensitivity, peak spatial frequency, and amount of contrast sensitivity attenutation (roll-off) at low spatial frequencies. Thus, rearing with esotropia produces effects on LGN X-cells with central receptive fields that are limited to spatial resolution.

5.2 MONOCULAR DEPRIVATION CAUSES EXPANSION OF X-CELL AND REDUCTION OF Y-CELL RETINOGENICULATE TERMINATIONS IN CATS. M. Sur*, A.L. Humphrey and S.M. Sherman. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

We recently used the technique of intracellular HRP injection into single X- or Y-cell optic tract axons to demonstrate their termination patterns in the lateral geniculate nucleus (LGN) of normal cats. X-cell axons innervate lamina A or A1 in narrow zones (100-170μm wide) oriented perpendicular to the geniculate lamination. Some X-cell axons also sparsely innervate the medial interlaminar nucleus (MIN) of the LGN. Y-cell axons innervate laminae C and A (from the contralateral retina) or lamina A1 (from the ipsilateral retina) in broad zones (200-400μm wide), and the majority also densely innervate the MIN.

Early monocular lid suture alters these termination patterns. We have successfully injected and recovered 9 X- and 13 Y-cell axons from deprived retinas as well as 11 X- and 7 Y-cell axons from nondeprived retinas. Physiologically, deprived and nondeprived axons have normal response properties. Morphologically, nondeprived axons are indistinguishable from normal axons. However, 4 deprived X-cells have LGN terminations that are broader (180-220μm) than normal and two of these have dense terminations in the MIN. Deprived Y-cell terminations seem even more abnormal. Four deprived Y-cells from the contralateral retina innervate only the C-laminae and do not innervate lamina A. Four additional deprived Y-cell axons have terminal fields in lamina A or A1 that are narrower (160-190μm) and sparser than normal. In general, Y-cell terminations are most affected in the A-laminae, less so in the MIN, and seem normal in the C-laminae. However, 2 deprived Y-cell axons that innervate the monocular segment appear to have normal morphology.

We have retrogradely labelled the somata of several injected axons. To date, there appear to be no qualitative morphological differences between deprived and nondeprived retinal ganglion cells, even though there are large changes in the terminal fields of the former.

The "expanded" X-cell termination zones and "shrunken" or "absent" Y-cell termination zones constitute the most peripheral morphological effects of deprivation discovered so far, and suggest two complementary developmental mechanisms: (1) binocular competition, and (2) competition between X- and Y-cell pathways. Thus, X-cells which seem to innervate the LGN before Y-cells do may have exuberant terminations that are "pruned" as Y-cells develop functional connections. Perhaps visual deprivation severely reduces the ability of Y-cell axons to dislodge the already present X-cell terminations in the A-laminae.

Supported by NIH Grant EY03038.

5.3 CORTICAL AXON TERMINAL ARBORIZATION AND SOMA LOCATION OF SINGLE, FUNCTIONALLY IDENTIFIED LATERAL GENICULATE NUCLEUS NEURONS. A.L. Humphrey, M. Sur* and S.M. Sherman. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

We have used the technique of intracellular HRP injection to label the terminal fields of single axons from the lateral geniculate nucleus (LGN) in areas 17 and 18 of the normal, adult cat. In addition, we have labeled the parent somata of the afferents in the LGN by retrograde transport of HRP from the intra-axonal injections in cortex. This allowed us to examine relationships between a cell's soma size and location within the LGN and the location and extent of its axon terminal field in cortex. LGN axons were recorded in the optic radiations beneath areas 17 and 18 using micropipettes (90-120 Mohm) filled with 3% HRP. Each axon was identified extracellularly as X or Y using a battery of tests, including conduction latency to optic chiasm stimulation and linearity of spatial summation. The axon was then impaled and HRP iontophoresed into it. During subsequent histology, cortical sections were reacted with diaminobenzidine and cobalt chloride to allow complete reconstruction of the injected axons, and the LGN sections were reacted with O-dianisidine to visualize the parent cells. Each cell was matched to its axon by retinotopic position.

There is a marked heterogeneity of termination patterns both within and between each afferent cell class and between areas 17 and 18. Axons of X-cells project only to area 17, while Y-cells project to area 17, to area 18 or to both areas. Axons of Y-cells in area 17 terminate in layers IVab, lower III and VI, while X-cell axons end mainly in layers IVc and VI, in agreement with Ferster and LeVay (J.Comp.Neurol.,182:923,1978). However, a number of X-cell fields spill over into lower IVab, indicating a marked overlap of X- and Y-inputs there. Within area 18, Y-cells terminate mainly in layers IV and IIIb, but some also issue collaterals into upper layer I. At least some of these Y-axons that project to layers IV plus I arise from cells in lamina C of the LGN which do not project also to area 17.

Retrogradely-filled cell bodies of injected axons have been found throughout the LGN. In the A-laminae, somata of X-cells range from 110 to 500μm^2, with a bias toward larger cells (>200μm^2), while those of Y-cells are 280-660μm^2. These ranges of values agree well with the sizes of intracellularly stained X- and Y-cells reported by Friedlander et al. (J. Neurophysiol., 46:80,1981) from recordings in the LGN, and they indicate that our micropipettes can record and inject axons of representative LGN cells. A geniculate neuron's soma size appears to be correlated generally with the width of its axon terminal field in visual cortex.

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5.4 THE EFFECTS OF MONOCULAR EYELID SUTURE ON THE TERMINAL ARBORIZATIONS OF PHYSIOLOGICALLY IDENTIFIED GENICULOCORTICAL AXONS. Michael J. Friedlander and Christiane Vahle-Hinz*. Dept. of Physiology and Biophysics, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Early monocular eyelid suture in kittens results in a severe reduction in the number of neurons in visual cortex which can be activated through the deprived eye. This deficit has been attributed to at least 2 possible mechanisms: 1) a decrease in the number of geniculocortical synapses from axons of cells in the lateral geniculate nucleus (LGN) laminae innervated by the deprived eye. (Shatz, C. J., and M. P. Stryker, 1978, J. Physiol. (Lond.), 281:267-283), and 2) the active suppression of geniculocortical input from the deprived eye. (Kratz, K. E., P. D. Spear, and D. C. Smith, 1976, J. Neurophysiol., 39:501-511; and Burchfiel, J. L., and F. H. Duffy, 1981, Brain Res., 206:479-484).

We have begun studies to test the first hypothesis. We are using the intracellular horseradish peroxidase (HRP) technique to physiologically classify and stain individual geniculocortical axons in cats that have been reared with monocular eyelid suture. Kittens are monocularly sutured at 6-9 days of age. Experiments are done on animals from age 9-15 months. Micropipettes are filled with a 3% HRP solution and bevelled to final impedances of 130-180 MΩ at 200 Hz. Stimulating electrodes are placed in the optic chiasm and optic radiations for transsynaptic and orthodromic activation of geniculocortical cells' axons, respectively. Axons are classified as X- or Y- based on a battery of physiological tests (Friedlander, M. J., C.-S. Lin, L. R. Stanford, and S. M. Sherman, 1981, J. Neurophysiol., 46:80-129) including response latency to stimulation of optic chiasm and the linearity of spatial summation within the axon's receptive field. The axon is then impaled with the micropipette. After verifying the axon's receptive field properties with intracellular recording, the axon is filled iontophoretically with HRP. The size, shape, laminar distribution, and density of the axon's terminal arborization in visual cortex are compared for X- and Y- axons from the LGN laminae innervated by the normal and the sutured eye. Preliminary data indicate that the terminal arborizations of axons from cells in the non-deprived LGN laminae occupy a greater volume of visual cortex than those from cells of the deprived laminae. In addition, the total number and density of apparent presynaptic terminals from the axons of the non-deprived laminae also appear to be greater than those from cells of the deprived laminae.

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5.5 GENICULATE Y-LIKE CELLS REMAIN AFTER MONOCULAR DEPRIVATION IN A PRIMATE. M. A. Sesma, T. Kuyk,* T. T. Norton, V. A. Casagrande, Depts. Anatomy and Psychology, Vanderbilt Univ., Nashville, TN; Dept. Physiol. Optics, School of Optometry, The Medical Center, Univ. of Alabama in Birmingham, Birmingham, AL.

Effects of early monocular lid closure in kittens and infant primates have been proposed by a number of investigators as a developmental model of human amblyopia. Deficits produced by this paradigm have been attributed to abnormal competitive interactions that, in cats, result in a reduction in recordable Y-cells (but not X-cells) in lateral geniculate nucleus (LGN) with correlated LGN cell size changes and form vision deficits (Sherman and Spear, '82). Similarly, in primates such as macaques and galagos, monocular suture produces profound changes in LGN cell size and associated visual deficits (Von Noorden et al., '76; Casagrande and Joseph, '80). However, in these primates the mean LGN cell size is altered in both the magno- and parvocellular laminae, suggesting that both Y- and X-like cells are affected by monocular lid closure.

We have examined this problem by recording from LGN neurons in 3 animals (Galago crassicaudatus), each deprived by monocular suture for more than 2 years. As before (Norton and Casagrande, '82), cell classification was determined using a battery of tests including linearity of spatial summation, latency to chiasm and cortical stimulation, receptive-field size, response to targets of appropriate standing contrast and phasic-tonic index.

In all 3 animals, deprived cells were easy to drive with visual stimuli and exhibited no gross abnormalities in receptive-field organization. Both linear and non-linear spatial summation were seen in the deprived Y-like cells of the magnocellular layer. Moreover, the encounter rate for deprived versus non-deprived neurons of all classes (W-, X- and Y-like) was the same for all layers regardless of whether retinal input was crossed or uncrossed.

These findings lead to two major questions. First, are the deprived LGN neurons of these primates really normal? We can so far only state that if physiological abnormalities exist in deprived Y-like cells or any other class in galagos, these effects seem quite subtle. Second, how can we reconcile the differences in deprivation effects on Y-cells in cats and galagos? The sensitivity of cat Y-cells to monocular deprivation may be a manifestation of the mixed retinal afferent input (both X and Y) to the same LGN laminae, a situation that would allow for direct competitive interaction within the LGN during development. This situation would be less likely to occur in primates where cell classes are segregated by layer, but still may compete at the cortical level. (Supported by 1-KO4-EY00223, EY01778, EY02909, EY03039, and NRSA fellowship EY05473.)

5.6 EFFECTS OF VISUAL DEPRIVATION ON THE GENICULOCORTICAL W-CELL PATHWAY IN THE CAT: AREA 19 AND ITS AFFERENT INPUT. A.G. Leventhal and H.V.B. Hirsch. Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132; Ctr. for Neurobiology, S.U.N.Y. at Albany, Albany, NY 12222.

We studied the receptive field properties of about 200 neurons in area 19 of normal cats and about 200 neurons in cats deprived of vision for 9-12 months by monocular lid suture. In these same animals we also studied the sizes of relay cells in the parvocellular C laminae of the dorsal lateral geniculate nucleus labeled by electrophoretic injections of horseradish peroxidase into area 19.

In area 19 of normal cats, the large majority of cells, regardless of laminar location and eccentricity, were binocular. Most responded equally well to the two eyes.

In area 19 of monocularly deprived (MD) cats, virtually all cells (97%), regardless of laminar location and eccentricity, responded only to stimulation of the normal eye. Thus, the effects of monocular deprivation upon area 19 are apparently more severe than those reported for area 17. In area 17 of MD cats, significant numbers of neurons in layer 4 can be activated by the deprived eye. Measurements of relay cells in the parvocellular C laminae labeled by injections into area 19 of deprived cats indicated that cell size in the deprived C laminae was unaffected by the deprivation even though cells in the deprived A laminae of these cats were severely shrunken.

These findings suggest that the types of cells found in the parvocellular C laminae (referred to collectively as W-cells) are not affected by visual deprivation in the same way as are the X- and Y-cells in the A laminae. Since laminar location is not related to binocularity in area 19 and the sizes of relay cells projecting to area 19 are not seriously affected by monocular deprivation, we suggest that binocular interactions in area 19 are mainly determined by intracortical connections.

5.7 NEUROGENESIS OF THE CAT'S PRIMARY VISUAL CORTEX. M.B. Luskin and C.J. Shatz. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

The distribution of radioactively labeled cells in the primary visual cortex of cats that received injections of ^{3}H-thymidine at different embryonic ages was examined in order to study the relationship between the time of neuron origin and the elaboration of afferent and efferent connections. To do so, 15 fetuses between embryonic day 17 (E17) and E59 (gestation is 65 days) received intrauterine injections (Hickey and Cox, Neurosci. Abst., 5:788, 1979) of 0.3-1.0 mCi of ^{3}H-thymidine and survived until 2 months postnatally before autoradiographic processing.

The earliest labeled cortical cells resulted from injections between E24 and E30. These cells were embedded in the white matter immediately below layer 6. In one fetus injected at E27 and allowed to survive only until birth, labeled cells were also observed in layer 1. However, in another fetus which received an injection at E28, but allowed to survive until postnatal day 56, virtually no labeled cells were found in layer 1, indicating that a transient population of cortical neurons may be present in the development of the cat's visual cortex.

Cells destined for the cellular layers of the visual cortex are generated between E28 and E56. Labeled cells were first seen within deep layer 6 following thymidine injections at E28 and E30. An injection at E35 labeled cells extending throughout layers 5 and 6, with the majority of labeled cells concentrated in the upper half of layer 6. Labeling within layer 4, the primary zone of termination of geniculocortical afferents, was first observed following an injection at E39, and was greatest in layer 4b. Injections at E46 through E56 labeled cells situated in layers 2 and 3. There was no apparent gradient in the rostro-caudal dimension or along the central to peripheral visual field representation. Thus, the overall pattern of visual cortical neurogenesis in the cat resembles that in the monkey (Rakic, Phil. Trans. R. Soc., 278: 245, 1977). In both species there is an inside-out gradient of development such that the earliest generated cells come to occupy the deepest layers and cells generated progressively later migrate to more superficial positions.

To further examine the relationship between the developing geniculocortical pathway and its target cells in layers 4 and 6, ^{3}H-proline was injected intraocularly in 8 fetuses aged E29 to E59, which were then allowed to survive 6 days. At E35 and E39 transneuronally transported label was observed in the optic radiations. This finding suggests that the earliest generated cells belonging to the white matter and layer 6 could serve as the first targets for ingrowing geniculocortical afferents. (Supported by NIH grants NS07158 to M.B.L. and EY02585 to C.J.S.)

5.8 THE DEVELOPMENTAL TOPOGRAPHY OF OCULAR DOMINANCE COLUMNS IN THE CAT STRIATE CORTEX. Martin S. Silverman* (SPON: Brenda Martin). Dept. of Physiology, Univ. of California, San Francisco, CA.

Using the ^{14}C-2-deoxyglucose (2DG) autoradiographic technique, we studied the developmental topography of ocular dominance (OD) columns in the cat striate cortex. Kittens between 10 and 39 days of age and adult cats were unilaterally enucleated under ether anesthesia. 2DG was injected 1 hr postoperatively, after which the cats viewed the laboratory environment for 1 hr. Subsequent 2DG processing included flat mounting the cortex before freezing, a modification that allowed almost the entire cat striate to be viewed in single tissue sections, obviating the need for extensive montage reconstruction of autoradiographic images.

The results showed that OD columns first appear between 12 and 15 days of age adjacent to the cortical representation of the temporal monocular crescent for the inferior visual field (situated at the anteroventral region of cat striate) and to the cortical representation of the optic disk (situated in the posterior region of cat striate). From 18 to 22 days of age, the OD columnar system expands from these two regions, completely covering the striate by 35 days of age. Comparison across hemispheres in individual cats showed that OD columns develop first and are more apparent contralateral to the intact eye. In addition, contralateral OD columns form a more confluent mesh-like pattern whereas ipsilateral OD columns are more dot-like. In both hemispheres of the adult, OD columns take a reticulated strip-like form that is intermediate in continuity to those seen during development. The adult patterns are seen to stream from the monocular crescent anteriorly and the optic disk representation in the posterior striate.

The initial formation and the final adult configuration of the OD columns suggest that the monocular crescent and the optic disk representations in striate act as primary initiators and organizers for the development of the OD columnar system. The monocular crescent and the optic disk representations are uniquely monocular areas of striate. The monocular/binocular interface that these areas both make with the adjacent binocular striate may be important for the induction of the OD columnar system in cat striate. (Supported by EY0014-12 and BNS 78-06171 to Russell L. De Valois)

5.9 DEVELOPMENT OF OCULAR DOMINANCE COLUMNS IN CATS REARED WITH BINOCULAR DEPRIVATION OR STRABISMUS. R.E. Kalil, Department of Ophthalmology, University of Wisconsin, Madison, WI 53706

In the cat, geniculocortical afferents representing each eye terminate in layer IV of visual cortex in patches that are partially segregated. The development of these patches has been studied in the normal cat (LeVay et al., J.Comp.Neur., 179, 1978) as has the influence of monocular deprivation (Shatz and Stryker, J.Physiol., 281, 1978). However, relatively little attention has been paid to the effects of other forms of visual deprivation or abnormal visual experience.

In the present experiments, cats were raised with binocular deprivation or misalignment of the eyes. In two cats, complete binocular deprivation was achieved by dark rearing (DR) from birth through 36 weeks. In two other cats the lids of both eyes were sutured shut (BD) at the time of eye opening. These cats were reared for six months, receiving only diffuse light stimulation during this period. Ocular misalignment was produced in two cats by cutting the lateral rectus muscle at 10 days postnatal. Both of these animals were raised to adulthood with a pronounced convergent strabismus.

Transneuronal autoradiography was used to study geniculocortical projections in each animal. Fluctuations in silver grain density, corresponding to the distribution of geniculocortical afferents, were quantified by counting individual grains at 40X. All counts were made in horizontal sections through area 17 ipsilateral to the injected eye along a distance of about 3.5 mm. At least two sections were counted from each brain. The sections that were selected showed most clearly a pattern of silver grains presumed to represent ocular dominance columns. When present, periodic fluctuations in grain density could be described by computing a peak to trough ratio (PTR = ave. max. density ÷ ave. min. density for 3 cycles). For reference, autoradiographic material from normal cats was available.

In DR and BD cats evidence for ocular dominance columns was weak. In most sections labelling in layer IV was almost uniform with little or no fluctuation in grain density. The best PTR for DR cats and for BD cats was about 2.0. By contrast, ocular dominance columns in cats with convergent squint were sharply delineated, with a best PTR of 4.8. The PTR for two normal cats was 2.9.

In comparison with normal cats the present results show that when binocular visual experience during development is partially or completely eliminated, geniculocortical afferents fail to segregate normally in area 17. Rearing with the ocular misalignment, however, produces the opposite effect, namely that the segregation of afferents representing each eye is enhanced.

(Special thanks to Mary Kay Ellis for assistance)

5.10 THE EFFECTS OF MONOCULAR DEPRIVATION ON THE PHYSIOLOGY AND ANATOMY OF THE KITTEN'S VISUAL SYSTEM. J. Presson and B. Gordon. Psychology Dept., University of Oregon, Eugene, OR 97403-1227.

Among the changes that occur in the visual system of a kitten reared with monocular lid suture are: (1) loss of responsiveness of visual cortex cells to stimulation of the deprived eye, (2) shrinkage of cells in the deprived laminae of the LGN, (3) reduction in the area occupied by deprived LGN terminals in layer IV of the visual cortex. Some investigators have suggested that one of these changes is primary and causes the others (Cynader and Mitchell, 1980; Cragg et al., 1976). If so, the primary change should occur first and should be a prerequisite to the others. To determine the sequence of these changes, we have looked for each of them after relatively short term deprivation. Kittens were deprived for 10 days beginning at 3 weeks of age, for 10 days beginning at 5 weeks of age, or for 20 days beginning at 8 weeks of age. Loss of responses to the deprived eye was assessed by recording from single units in the visual cortex. Shrinkage of LGN cells was assessed by comparing the estimated cross sectional area of cells in the deprived and nondeprived layers in Nissl stained sections. Reduction in the area of layer IV occupied by deprived LGN terminals was assessed with transneuronal autoradiography following injection of tritiated proline into the deprived eye. After 10 days of deprivation begun at 3 weeks of age the deprived eye was virtually unable to drive cortical cells and the LGN cells had shrunk by 21%; however transneuronal autoradiography showed that layer IV was virtually continuously labelled. Ten days of deprivation begun at 5 weeks produced similar changes in ocular dominance and LGN cell size, but in these animals the geniculate terminals had segregated and the portion of layer IV occupied by terminals from the deprived eye was abnormally small. Twenty days of deprivation beginning at 8 weeks of age resulted in a loss of cortical responses to the deprived eye, but the effect was not as great as in the younger animals. These older animals showed minimal LGN cell shrinkage, about 9%. The reduction in the area of layer IV occupied by deprived LGN terminals was, however, similar to that in the animal deprived at 5 weeks. These results suggest that changes in the ocular dominance distribution do not require changes in the anatomic distribution of geniculate terminals, and that changes in terminal distribution can occur in the absence of marked LGN cell shrinkage. The possibility exists, however, that minute, but functionally significant, terminal retraction might not be visible in the light microscope; or that very small changes in LGN cell size might cause changes in terminal distribution. (This work was supported in part by the Medical Research Foundation of Oregon.)

5.11 VISUAL CORTICAL PLASTICITY: DEFICIT AFTER ACUTE, BUT NOT CHRONIC, NORADRENERGIC DENERVATION WITH 6-HYDROXYDOPAMINE. Mark F. Bear*, Michael A. Paradiso* and J. D. Daniels. Center for Neural Science, Brown University, Providence, RI 02912

Kasamatsu, Pettigrew, and coworkers have reported that depletion of cortical catecholamines by either intraventricular or intracortical administration of the neurotoxin, 6-hydroxydopamine (6-OHDA), renders visual cortex largely unresponsive to monocular deprivation. A convenient means to reliably deplete cortical norepinephrine (NE) is the systemic administration of 6-OHDA to neonates. We elected to try this approach in an attempt to test the idea that the normal noradrenergic innervation of cortex is required for developmental plasticity.

13 kittens received intraperitoneal injections of 6-OHDA (200 mg/kg) on postnatal days 1 and 2. Littermates served as vehicle-injected controls. At about one month of age, the kittens were monocularly deprived, and 10 days later, were prepared for extracellular recording from area 17. Following the recording session, visual cortical tissue was rapidly dissected, frozen and prepared for high pressure liquid chromatography (HPLC). The biochemistry confirmed the effectiveness of the drug treatment: injected animals had less than 11% of control NE. However, despite this marked depletion of NE, visual cortex responded normally to monocular lid closure with a fully-shifted ocular dominance histogram.

This result prompted us to try to repeat Kasamatsu and Pettigrew by following their exact 6-OHDA delivery procedure. 10 kittens, normally reared to 6-8 weeks of age, were monocularly deprived and fitted with osmotic minipumps and cannulae leading to visual cortex. 6-OHDA (1mg/ml) was delivered to area 17 of one hemisphere (1 ul/hr), vehicle solution to the other. Blind recordings 7 days later, revealed that the 6-OHDA perfused hemisphere was much less affected by monocular deprivation than the control. Again, biochemistry confirmed NE-depletion in the drugged cortex.

In summary, we observe that the chronic removal of cortical NE leaves visual cortical plasticity intact, while acute NE depletion causes a deficit in plasticity. We conclude: first, that some form of compensation for the lost NE occurs in kittens chronically depleted from birth; second, that a simple correlation of NE levels with plasticity is insufficient to account for all the developmental capabilities of kitten visual cortex.

We acknowledge the support of ONR contract N00014-81-K-0136.

5.12 THE ONTOGENETIC CHANGES IN METABOLISM OF cAMP IN THE VISUAL CORTEX OF CATS. C. Aoki* & P. Siekevitz. Dept. of Cell Biology, The Rockefeller University, New York, N.Y. 10021.

The evidence from many labs indicate that the neuronal plasticity of the postnatally developing visual cortex is dependent on visual experience.[1,2] Recently, Kasamatsu et.al. have reported evidence indicating that this form of plasticity is influenced by the local concentration of l-norepinephrine (NE) in the visual cortex[3,4,5,6] Further, Cynader's group has reported that the plastic period, which spans the first 3 to 4 months after birth, can be postponed at least a year, so long as the animal is completely deprived of visual experience during that time[7,8] Our goal was to study the molecular mechanism by which NE modulates visual cortical plasticity of this type. We have examined the ontogenetic changes in adenylate cyclase(ACase) specific activity which is stimulated by NE, as well as cAMP- and cGMP-phosphodiesterase(PDE). The ontogenetic changes in the enzymes' specific activities were then compared with those tissues obtained from kittens dark-reared for various lengths of time(donated to us by M Cynader's laboratory), so as to discriminate genetically determined developmental changes from changes dependent on visual experience. cAMP- and cGMP-PDE in area 17 of normally reared cats rise acutely in specific activity postnatally and plateau to adult level around the 2nd month. The levels of these enzymes were not affected by the dark-rearing condition. ACase specific activity of area 17 of normally reared cats is very low soon after birth, increases dramatically between 2nd and 4th week, and plateaus to adult level at the 3rd month. At all ages, a stimulation by NE and GTP was observed, and this stimulation by the hormone was greater during the critical period than at ages before or after the period. We are currently examining the ACase activity in dark-reared kittens.

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5.13 RECOVERY OF BINOCULAR CONNECTIONS IN CAT VISUAL CORTEX.

Rafi Malach*, Robert Ebert* and Richard C. Van Sluyters. School of Optometry and Neurobiology Group, University of California, Berkeley, CA 94720.

We have examined recovery from the physiological effects of visual deprivation in kittens subjected to a total of 12 hr of monocular deprivation (MD) distributed over 2 days (three, 2-hr periods each day) at 4 wks of age, and then allowed various periods of binocular vision. Single units were recorded from the striate cortex of these kittens to assess the ocular dominance of cortical neurons at different stages of recovery. Recordings taken from 8 kittens immediately following the period of MD revealed that ocular dominance was shifted heavily in favor of the non-deprived eye and that the proportion of binocular units was reduced to about 25% of the visually responsive cells sampled. After allowing 3 wks of binocular vision, we recorded a second time from 6 of these kittens, and the pattern of ocular dominance had changed substantially. Now, the two eyes dominated roughly equal numbers of cells and the proportion of binocular neurons had increased to about 65%. Four of these kittens were given an additional 4 wks of binocular exposure, and a third recording session revealed an additional small increase in the relative number of binocularly driven neurons.

To examine somewhat further the speed with which the recovery process occurs, we recorded from another group of 6 kittens that were monocularly deprived for 12 hr and then allowed an identical period of binocular exposure. After 12 hr of binocular vision, the deprived eye had re-established functional connections with about half of the cells studied, but the proportion of binocular units was still substantially below that seen after 3 wks of recovery. Our results demonstrate that binocular visual experience can bring about a rapid and essentially complete recovery of functional cortical connections from the deprived eye in kittens subjected to a brief period of MD early in life. Possible mechanisms underlying this recovery process will be discussed.

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6.1 DEVELOPMENT OF INNERVATION OF ARTERIES AND ARTERIOLES OF THE RAT MESENTRY. D.F. van Helden*, C.E. Hill* and G.D.S. Hirst* (SPON: B. Walmsley). Department of Pharmacology, John Curtin School of Medical Research, Australian National University, Canberra, ACT, 2601, Australia.

Here we report on the development of innervation of rat mesenteric arteries (diam. 200-300 μm) and arterioles (diam. 80-150 μm). Thirty two rats aged 1-21 days postnatally were used. In vitro intracellular recordings were made from the smooth muscle layer and responses to nerve stimulation examined. Perivascular nerve stimuli (pulse width .01-.1 ms, amplitude 10-100V) were applied using a suction electrode at the proximal end of the mesenteric artery.

Slow depolarizing potentials were often recorded from both arterioles and arteries even from the youngest animals examined. These were occasionally detected following a single stimulus but more often a train of stimuli was required. Excitatory junction potentials (EJPs) which could be summed by a train of stimuli to initiate an action potential, were first recorded in the arterioles of day 9 animals. However, EJPs could not be recorded from the more proximal arteries until at least day 12. EJPs recorded from these immature animals showed large variance in both amplitude and rise time with larger responses having faster rise times. This finding was consistent with the prediction from cable theory for a system with point current sources (synapses) distributed at low density over the cable (arteriole), with each source having a low probability of activation. Fluctuations of EJP amplitude and rise time diminished over the next 4 to 7 days and by about day 18 both arterioles and arteries had relatively uniform EJPs.

Using fluorescence microscopy, nerve fibres were detected running along the arteries and then branching sparsely on the arterioles as early as day 1. The density of this innervation increased with age, dense plexuses developing some 2 to 4 days earlier in the arterioles than in the arteries.

Taken together these data suggest that sympathetic nerves have discrete target zones within an artery/arteriolar tree.

6.2 EFFERENT SPLANCHNIC ACTIVITY IN NEONATAL SWINE. P.M. Gootman, H.L. Cohen, S.M. DiRusso*, A.P. Rudell and L.P. Eberle*. Dept. Physiol., Downstate Med. Ctr., SUNY, Brooklyn, N.Y. 11203.

Studies of postnatal neural control of the circulation (Gootman, et al., In Ciba Symposium #83 "Development of the Autonomic Nervous System", 1981, pp. 70-93, Fed. Proc., 1983, 42) have shown different rates of maturation for cardiovascular reflexes. Because the renal and superior mesenteric vascular beds are quite responsive in the neonate, we decided to examine the spontaneous efferent discharge of the major preganglionic sympathetic supply to these beds, the greater splanchnic nerve (SPL). Animals ranging in age from birth to 2 weeks of age were lightly anesthetized with halothane in O_2, immobilized with decamethonium Br and artificially ventilated to maintain normal arterial blood gases and pH. The SPL was located retroperitoneally and subdiaphragmatically, cut just proximal to the celiac ganglion and, in piglets 1 week or older, desheathed. Monophasic recordings were made with a bandpass of 0.1-1000 Hz and stored on magnetic tape along with simultaneous recordings of blood pressure, EKG and, in selected experiments, efferent phrenic nerve activity (monitor of the central respiratory oscillator). Off-line analyses involved computer averaging, correlation and power spectra techniques. At present, three types of periodicities have been observed: 1) 7-17/sec waves, 2) oscillations in phase with cardiac cycle, and 3) oscillations in phase with the central respiratory cycles (maximum activity during inspiration and minimum activity in early expiration). SPL activity in piglets less than 1 week of age, was found to be particularly sensitive to body temperature. A decrease in body temperature below 37°C, i.e., a change in 1°C, resulted in loss of SPL discharge. The results of these experiments indicate that tonic sympathetic discharge to the celiac ganglion and adrenal medulla is present in piglets at birth with periodicities similar to those seen in adult SPL (Cohen and Gootman, Am. J. Physiol., 1970, 218: 1092; Gootman and Cohen, Am. J. Physiol., 1970, 219: 897, J. Autonomic Nervous System, 1981, 3: 379; Gootman et al., Brain Res., 1975, 87: 395). The presence of SPL activity could account for the marked changes in renal and superior mesenteric blood flows observed during either afferent or central stimulation.
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6.3 NEURAL CREST-MICROENVIRONMENT INTERACTIONS IN THE FORMATION OF ENTERIC GANGLIA: AN ANALYSIS OF NORMAL AND LETHAL SPOTTED MUTANT MICE. T.P. Rothman, G. Nilaver and M.D. Gershon. Dept. Anatomy and Cell Biology and Dept. Neurology, Columbia Univ. P&S, NY, NY 10032.

The l^s/l^s (lethal spotted) mutant mouse develops megacolon proximal to a 2-3mm terminal segment of aganglionic bowel. The current experiments were done to determine whether this aganglionosis is due to a slowed transit down the gut of ganglion cell precursors from the rhombencephalic neural crest, an abnormal microenvironment in the aganglionic zone that is not conducive to the growth of intrinsic enteric neurons, or to a secondary degeneration of precursors that initially invade the terminal bowel. Segments of control (E9-E20) and l^s/l^s (E10-E20) gut were explanted and grown in organotypic tissue culture for 2 weeks. Neurons that contain acetylcholinesterase (AChE), substance P (SP), vasoactive intestinal polypeptide (VIP) or serotonin (5-HT) immunoreactivity were found in control explants, even from the terminal bowel, as early as days E9-E11. In situ, AChE and 5-HT-containing neurons appear first in the foregut on E12 and reach the terminal colon by day E14. SP- and VIP-containing neurons appear in the foregut in situ on day E14. Explants removed between E9 and E12 thus contain neuronal precursors that can give rise to at least 4 types of enteric neuron in vitro. These precursors, in control mice, reach the hindgut by day E9. Explants from l^s/l^s gut behave as control explants except for tissue removed from the terminal 2-3mm of bowel. Segments from this region at any age remain aneuronal in culture. The length of presumptive aganglionic bowel in fetal l^s/l^s mice is the same from E11 through birth and roughly corresponds to the length of the aganglionic segments of adults. In adults, the aganglionic region receives an innervation by axons containing SP, VIP, and AChE. These are probably extrinsic; however, the entirely intrinsic 5-HT-containing fibers are absent.

These studies indicate that the proximo-distal sequence of enteric neuronal phenotypic expression is not related to the rate of migration of precursors down the gut since the precursors reach the hindgut at the same time as they reach the foregut. No evidence was obtained to indicate that migration is slower than normal in l^s/l^s animals. Removal of presumptive aganglionic bowel to tissue culture fails to rescue enteric neurons. Results are consistent with the hypothesis that the microenvironment of the terminal 2-3mm of l^s/l^s bowel is abnormal and does not support its colonization by precursors of intrinsic enteric neurons or its innervation by intrinsic enteric axons. Supported by grants BNS79-11640, NS15547 and MOD 1-747.

6.4 EMBRYONIC DEVELOPMENT OF THE RAT SUPERIOR CERVICAL GANGLION. Eric Rubin* (SPON: C.J. Forehand), Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

Sympathetic ganglia are simple systems open to a variety of approaches for the study of neural development. I report here physiological and morphological findings which show the rapid development of several aspects of innervation in the rat superior cervical ganglion.

On embryonic day 11 (E11; conception = E0; birth = E22), no distinction can be made between the prospective superior cervical ganglion and its preganglionic nerve based on light microscopy (silver staining) or at the ultrastructural level; instead, a uniform cylinder of intermixed cells and axons extends through the cervical region, dorsal to the carotid artery. By E14, however, the ganglion has coalesced, and the pre- and postganglionic nerves are apparent.

On E15, the ganglion shows the following features. Restriction of preganglionic supply: Horseradish peroxidase (HRP) applied to the preganglionic nerve labels neurons in spinal segments T1-T5 (primarily T1-T3), the same segments as innervate the adult ganglion. Ganglionic synapse formation: Junctions with ultrastructural features typical of mature ganglionic synapses are present. Cholinergic transmission: Preganglionic stimulation evokes a two-component response in postganglionic nerves. The longer latency component of this response fatigues readily and is blocked by curare and by low Ca^{++}/high Mg^{++} Ringer. A similarly labile, but much smaller, response occurs on E14. Dendrites: Ganglion cells backfilled with HRP display several prominent dendrites. Axonal extension: The axons of some ganglion cells are in the vicinity of distant targets, since these neurons are labelled by HRP injected into the eye.

Thus, by E15 the superior cervical ganglion is already well organized. Such prompt maturation emphasizes that certain aspects of this system, such as the formation of specific synaptic connections, may originate during the earliest stages of ganglion development.

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6.5 DEVELOPMENT OF INTRINSIC SYNAPSES IN THE RAT SUBMANDIBULAR GANGLION. K. Kawa* and S. D. Roper. Departments of Anatomy and Physiology, University of Colorado Health Sciences Center, Denver, CO 80262.

The submandibular ganglion in the rat is a good preparation for investigating neuron-target interactions. The organization of neurons and their synaptic connections is simple, and there are no interneurons in the ganglion. The target organs, the salivary glands, can readily be manipulated pharmacologically or surgically and the effects of such manipulations tested on the development and maintenance of neurons in the ganglion. Further, the submandibular ganglion is very thin and individual cells and their processes can be seen in the living isolated preparation.

In our ongoing investigations of the development and maintenance of synapses in the submandibular ganglion, we have discovered that there are excitatory synaptic connections between ganglion cells. These intrinsic ganglionic synapses occur both in newborn and adult animals.

To test for the presence of intrinsic synapses in the submandibular ganglion, rats were anesthetized, the chorda tympani nerve (which carries the preganglionic nerve supply) was severed at the middle ear, and the animals were allowed to recover for 3 days so that all extrinsic innervation to the ganglion degenerated. Submandibular ganglia were then removed and pinned out in a recording chamber, neurons impaled with glass micropipettes, and postganglionic axons stimulated with a suction electrode. Intrinsic synapses were readily distinguishable from antidromically-propagated action potentials. For example, intrinsic synaptic responses fluctuated in quantal steps when stimulated at frequencies above 1 Hz.

There was a regional distribution of intrinsic synapses in the adult rat. Neurons which innervated the submaxillary salivary gland tended to lie along the salivary ducts in large clusters and the incidence of intrinsic synapses was high in these clusters. Ganglion cells which innervated the sublingual salivary gland lay individually or in small clusters on the thin sheet of connective tissue which spans the gap between the preganglionic nerve supply and the salivary ducts. The incidence of intrinsic connectivity in these latter neurons was low.

We are currently investigating (a) whether the incidence of intrinsic synapses is determined more by the location of the ganglion cell or by the location of its target, and (b) how the incidence of intrinsic synapses changes as the rat matures from newborn to adult. These investigations were supported in part by NIH grant NS11505.

6.6 DEVELOPMENTAL CHANGES IN THE NEUROTRANSMITTER PROPERTIES OF CHOLINERGIC SYMPATHETIC NEURONS IN VIVO. R. E. Siegel*, M. Schwab* and S. Landis. Dept. of Neurobiology, Harvard Med. Sch., Boston, Mass. 02115 and Max Planck Institute for Psychiatry, Munich, Ger.

Neurons dissociated from sympathetic ganglia of newborn rats can be induced to undergo a transition from adrenergic to cholinergic function when grown in certain culture conditions. Examination of a cholinergic sympathetic system in vivo, the neurons innervating sweat glands (SGs) in rat footpads, has suggested that a similar transition in neurotransmitter expression takes place during normal development. While such markers of adrenergic function as catecholamine fluorescence and small granular vesicles are found in all fibers innervating SGs of 7 day postnatal rats, these markers are no longer detectable by 21 days.

We have used immunocytochemical techniques to obtain further evidence for transmitter plasticity in neurons innervating SGs. The properties of both the SG fibers and their cell bodies of origin have been characterized. At 7 days, nerve fibers possessing tyrosine hydroxylase (TH) and dopamine-B-hydroxylase (DBH) immunoreactivity are present around all SGs. Between 10 and 21 days, the intensity of the fiber staining decreases considerably but does not entirely disappear. In contrast, vasoactive intestinal peptide (VIP), a neuropeptide which has been localized in cholinergic but not adrenergic sympathetic neurons in the cat (Lundberg et al., 1982, PNAS 79:1303), and acetylcholinesterase (AChE) increase over the same time course. Histochemical staining for AChE appears around a few SGs at 7 days and VIP-like immunoreactivity is first detected 1 or 2 days later. By 21 days the plexus of SG fibers stains intensely for both markers.

The cell bodies of origin for the SG fibers of the front feet are thought to lie in the stellate ganglion. In the mature stellate, approximately 3% of the cells possess VIP-like immunoreactivity. If fluorescein-conjugated wheat germ agglutinin is injected into the front foot pads, a small proportion of these VIP immunoreactive cells are also retrogradely labeled. The target tissues of the remaining VIP positive cells are unknown. A majority of the VIP immunoreactive cells do not possess TH immunoreactivity, a finding consistent with the coexistence of acetylcholine and VIP in certain sympathetic neurons. However, almost all of the VIP positive cells do contain DBH immunoreactivity. These properties of the cell bodies, in conjunction with the changes observed in SG fibers during development, indicate that adrenergic neurons in vivo can become cholinergic while still retaining some adrenergic characteristics. Further studies are being performed to examine how the properties of individual neurons projecting to the SGs change during development. Supported by NINCDS and the American Heart Association.

6.7 EXPRESSION AND DEVELOPMENT OF THE ADRENERGIC PHENOTYPE IN RAT MEDULLA OBLONGATA. M.C. Bohn, M. Goldstein and I.B. Black. Division of Developmental Neurology, Cornell Univ. Med. Coll., New York, N.Y. 10021 and Dept. of Psychiatry, New York Univ. Medical Center, New York, N.Y. 10016.

We have previously reported that the epinephrine-forming enzyme, PNMT (phenylethanolamine-N-methyltransferase), a specific index of the adrenergic phenotype, initially appears in rat adrenal medulla and sympathetic ganglia on E17 (embryonic day 17). Moreover, the subsequent development of PNMT in these neural crest derivatives is regulated by glucocorticoids. We have now investigated the initial expression and development of PNMT in the medulla oblongata to determine whether adrenergic expression in brain and periphery are similarly regulated.

PNMT-containing cells were initially observed in the medulla by immunofluorescence at E14. By E15, the number of PNMT cells and the intensity of immunofluorescence had increased dramatically. Isolated cells with long beaded processes were located within the subventricular zone while clusters were observed ventrolaterally in the presumptive C1 region. Other cells appeared to be migrating from the ventricular zone to the C1 region, along a path rich in PNMT containing fibers. In the term embryo, PNMT cells were grouped in presumptive C1, C2 and C3 regions, as described in the adult rat (Howe et al, Neurosc. 5, 2229, 1980), although there still appeared to be PNMT cells in the process of migration.

PNMT catalytic activity was also assayed in the medulla to assess adrenergic development. PNMT activity was initially detectable on E14, and increased over 30-fold, reaching adult plateau values at 20 days. To evaluate the possible role of glucocorticoids in brain PNMT ontogeny, bilateral adrenalectomy was performed on the first day of life. Adrenalectomy did not alter the development of medullary PNMT. Moreover, treatment of pregnant rats with dexamethasone (1mg/Kg q.d.), from E14 to term did not alter enzyme activity in newborns. Finally, adrenalectomy in the adult did not alter medullary activity during the postoperative month.

Our observations suggest that there are marked differences between brain and periphery in expression and development of the adrenergic phenotype. PNMT is expressed in embryonic brain 3 days earlier than in the periphery, suggesting that adrenergic expression is not simultaneously triggered by a common signal in all regions of the embryo. Further, the ontogeny of PNMT in brain does not appear to be glucocorticoid dependent, whereas sympathoadrenal PNMT is critically dependent on the hormones. (Supported by NIH grants NS 00713, NS 18420, NINDS 06801, NS 10259, HD 12108 and by the Familial Dysautonomia Foundation).

6.8 THE EFFECT OF COPPER SUPPLEMENTATION ON THE CONCENTRATION OF COPPER AND THE ACTIVITY OF DOPAMINE-beta-HYDROXYLASE IN THE BRAIN OF THE BRINDLED MOUSE. G. L. Wenk and K. Suzuki. Department of Pathology/Neuropathology, Albert Einstein College of Medicine, The Bronx, NY 10461.

The brindled mutant mouse is a useful model to study Menkes Kinky Hair Syndrome. The metabolic dysfunctions in both humans and rodents are related to insufficient levels of bioavailable copper (Cu). The abnormal Cu distribution is thought to be due to the presence of significantly increased amounts of cysteine-rich 10,000 dalton Cu-binding protein (Labadie et al., Pediatr. Res. 15:257,1981). Recently Cu supplementation therapy has been able to both prevent the appearance of various neuropathological changes and prolong the life of these mutant mice (Nagara et al., J. Neuropath. Exp. Neurol., 40:428,1981). The optimum conditions for supplementation have been shown to be two intraperitoneal injections on post-natal days 7 and 10. This presentation reports on the brain Cu concentrations prior to, during, and after the same day 7 and 10 Cu therapy. The mice were sacrificed on various days and the brains were wet digested in nitric acid and the Cu concentrations were determined by atomic absorption spectrometry using a graphite furnace. The results show that post-natal days 7 and 10 correspond to two important epochs in Cu homeostasis. The supplementation therapy seems to provide sufficient bioavailable Cu to respond to the needs of the mice at these crucial times.

We also investigated the effect of the day 7 and 10 Cu therapy on the activity of the Cu-dependent enzyme dopamine-beta-hydroxylase (DBH). The DBH was assayed according to a method by Borchardt et al., (Anal. Chem., 51:1960,1979). The results follow the changes in DBH activity which occur when these mice are Cu-deprived, and then are given additional Cu. An improvement in DBH activity may be due to the increased availability of Cu to be used in the enzyme complex, or to the ability of Cu to inhibit various endogenous DBH inhibitors known to be present in the brain.

We have compared the in vitro activity with and without Cu added to the media, and the in vivo activity, by measuring endogenous norepinephrine and dopamine levels in separate aliquots of tissue homogenate.

6.9 EXPRESSION OF CATECHOLAMINERGIC CHARACERISTICS BY PERIPHERAL SENSORY GANGLION CELLS IN THE NORMAL ADULT RAT IN VIVO. D.M. Katz, K.A. Markey, M. Goldstein and I.B. Black. Div. of Developmental Neurology, Cornell Univ. Med. College, 515 E. 71st St., New York, New York 10021 & Dept. of Psychiatry, New York University Medical Center, New York, N.Y. 10016.

Recent work demonstrating the extreme lability of neurotransmitter phenotype in sympathetic neurons calls into question the identification of functional classes of neurons on the basis of individual phenotypic characteristics. In order to further examine relationships among neuronal phenotypic traits, we are studying the in vivo expression of catecholaminergic characteristics by sensory ganglion cells in the normal adult rat. Using a highly specific and well-characterized antibody against tyrosine hydroxylase (TOH) we have identified neurons in the nodose and petrosal ganglia that exhibit abundant TOH immunoreactivity. In addition, some ganglion cells exhibit faint formaldehyde-induced fluorescence, and this fluorescence is enhanced by pretreatment of animals with pargyline (50 mg/Kg, i.p.). Pargyline inhibits the enzyme, monoamine oxidase, that is responsible for the intraneuronal metabolism of catecholamines. The TOH-containing cells possess morphologic characteristics typical of primary sensory neurons, including an initial axon glomerulus and a single bifurcating neurite. These cells are readily distinguishable from TOH-containing SIF cells by their size, morphology, and staining characteristics. Furthermore, the TOH-containing neurons appear to be insensitive to neonatal treatment with 6-Hydroxydopamine, thereby distinguishing them from sympathetic neurons. Using a sensitive radiochemical assay we are able to measure TOH catalytic activity within the nodose and petrosal ganglia. Peripheral axotomy reduces specific TOH activity within the ganglia and depletes neuronal TOH immunoreactivity.

Our data indicate that some adult sensory ganglion cells normally express catecholaminergic phenotypic characteristics in vivo. Classically, the catecholaminergic phenotype has been associated, in the peripheral nervous system, with sympathetic neurons. The existence of sensory ganglion cells that exhibit catecholaminergic neurotransmitter traits and morphologic characteristics typical of sensory neurons suggests that the ability to express the catecholaminergic phenotype is not restricted to any particular functional class of peripheral neurons. (Supported by NIH post-doctoral fellowship NS06623-01 to D.M.K., and NIH Grants NS10259 and HD12108.)

7.1 DIFFERENT POPULATIONS OF GRANULE CELLS INNERVATE SUPERFICIAL AND DEEP SUBLAMINAE OF THE EXTERNAL PLEXIFORM LAYER OF THE RAT OLFACTORY BULB. Edward Orona and John W. Scott, Department of Anatomy, Emory University, Atlanta, Georgia 30322.

The basal dendrites of tufted cells of the main olfactory bulb ramify in the superficial half of the external plexiform layer (EPL), while those of most mitral cells are confined to the deep half of the EPL. The response properties of mitral and tufted cells are modulated by bulbar interneurons, including the granule cells. We report here that there may be different populations of granule cells with separate but overlapping projections.

Small, extracellular injections of horseradish peroxidase (HRP) were made iontophoretically (4% HRP) into the superficial and deep parts of the EPL and of the granule cell layer (GCL) in rats. Superficial EPL injections labeled principally superficial granule cell somata, whereas deep EPL injections additionally labeled deep granule cell somata. Injections deep in the GCL labeled granule cell dendritic processes extending only into the deep half of the EPL. In contrast, following superficial GCL injections, granule cell dendrites were observed extending across the entire EPL. These results were the same in material processed with the Hanker-Yates procedure where we could follow individual dendrites and in the more sensitive tetramethyl benzidine procedure.

It appears that tufted cells are innervated only by superficial granule cells. We cannot tell from this material whether the mitral cell dendrites of the deep EPL are contacted exclusively by deep granule cells or by the total population of granule cells. Since tufted cells differ from mitral cells in their responses to stimulation of the olfactory nerve (e.g. Schneider and Scott, Neurosci. Abstracts #217.5, 1981), these properties may be partly determined by the differences in granule cell innervation. Furthermore, centrifugal afferents terminate in distinct sublaminae of the GCL and could differentially affect these populations of granule cells (Davis et al. JCN, 204:475, 1981). Other recent reports also suggest that the granule cell layer has a laminar distribution of cell types (Davis et al. JCN, 204:377, 1982; Struble and Walters, Brain Res., 236:237, 1982). Our data are consistent with the hypothesis that mitral and tufted cells function differently in processing olfactory information and that their responses may be modulated by different populations of granule cells.

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7.2 ULTRASTRUCTURAL ORGANIZATION OF THE OLFACTORY BULB FOLLOWING LOSS OF MITRAL CELLS IN THE MUTANT MOUSE PCD. Charles A. Greer, Norbert Halasz* and Gordon M. Shepherd, Section of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

It has been found that a neurological mutant mouse, Purkinje Cell Degeneration (PCD), loses greater than 80% of its olfactory bulb (OB) mitral cells by 4 mon. postnatal and 96% by 8 mon. (Greer & Shepherd, Brain Res., 235: 145, 1982). This extraordinarily large and selective loss of a single cell type does not appear to affect the afferent input to the OB, as judged by mapping odor-induced activity with the 2-deoxyglucose method. Normally, the mitral cells, together with the tufted cells, are the main output neurons of the OB. Both cells are also intrinsic components of local circuits within the OB by means of dendrodendritic synaptic interactions with interneurons. We have therefore examined the ultrastructure of the PCD OB to assess the effect of mitral cell loss upon local circuit organization.

Affected, homozygous recessive, PCD mice and their normal, pcd/++, littermates were studied. The mice received an intracardiac perfusion of phosphate buffered 1% paraformaldehyde and 2.5% glutaraldehyde and their olfactory bulbs were processed for electronmicroscopy. The tissue was examined in both randomly chosen areas of approximately 130 um^2 and in montages of approximately 4000 um^2.

Examination of the electronmicrographs revealed in the homozygous recessive PCD mice a decrease in the frequency of large electron luscent dendritic profiles, characteristic of mitral cell dendrites, within the external plexiform layer. Smaller pale profiles, attributed to tufted cell dendrites were easily recognized as were the electron dense dendritic spines of granule cells. In addition, within the external plexiform layer, polarized synaptic contacts including Gray Type I (tufted to granule) and Gray Type II (granule to tufted) were observed both singly and in reciprocal dendrodendritic pairs.

Thus far the data suggest that the synaptic organization of tufted and granule cells is maintained following the loss of mitral cells although further quantitative analyses may reveal changes in the ratios of synaptic types. In addition to the difference between mitral and tufted cells in their control by the genome as suggested by these data, recent reports have indicated that the central projections of these two neurons differ (Scott et al., JCN, 194: 519, 1980). Therefore, PCD may also prove useful for studying aspects of plasticity and synaptic reorganization in the basal forebrain in parallel with those of the local circuits in the OB.

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7.3 ANATOMICAL EVIDENCE FOR A CONVERGENCE OF OLFACTORY AND GUSTATORY-VISCERAL AFFERENT PATHWAYS IN MOUSE CEREBRAL CORTEX. Y. Geinisman, M.T. Shipley, and J.F. Disterhoft. Dept. Cell Biol. & Anat., Northwestern Univ. Med. Sch., Chicago, IL 60611

The perception of flavor depends on both olfactory and gustatory, and possibly visceral afferent (VA) sensations. The functional association among these distinct modalities must be mediated in the brain since the first order pathways for olfaction and gustatory-VA are widely separated. However, olfactory and gustatory-VA circuits have no known sites of convergence in the CNS. We report anatomical data which suggest that these modalities converge in the mouse cerebral cortex after traversing surprisingly few synaptic relays.

We have examined the connections of the mouse main olfactory bulb (MOB) using ortho- and retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). We confirmed (Neurosci. Abst., 1981, 7:731) afferent and efferent connections characteristic of the rodent MOB. In addition, we found strong orthograde labeling in the insular cortex (IC), dorsally adjacent to the piriform cortex (PC). To determine whether this labeling pattern represented a terminal projection, electron micrographs of PC were compared with ones of IC 3 days after a complete MOB transection. Degenerating presynaptic terminals in IC were indistinguishable from those in PC, in morphology and location.

The inputs and outputs of this MOB-recipient insular field were studied with small (8-10 nl) WGA-HRP injections. The results demonstrate that this IC sector has strong connections with every subcortical structure associated with the ascending gustatory-VA pathways. Massive inputs to IC were found from the gustatory-VA part of the ventrobasal thalamus (VB) and there were direct inputs from the second order gustatory relay, nucleus parabrachialis (NPB). Outputs of IC were traced to VB, NPB, the central nucleus of the amygdala and (heavily) to the first order gustatory-VA relay, the nucleus of the solitary tract. In addition there were reciprocal connections with the olfactory part of the medial dorsal thalamic nucleus.

These data suggest that the MOB has a direct terminal projection to the primary sensory cortex for the gustatory and VA systems. Thus, olfactory and gustatory-VA pathways appear to converge at a surprisingly early stage of synaptic processing. This inter-modal convergence area in IC may play a key integrative role in flavor perception, visceral feedback and learned tastes. The neurophysiology of this cortical area is currently under investigation.

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7.4 DEVELOPMENT OF A FUNCTIONALLY AND MORPHOLOGICALLY SPECIALIZED REGION OF THE RAT OLFACTORY BULB: THE MODIFIED GLOMERULAR COMPLEX. William B. Stewart, Charles A. Greer, Patricia E. Pedersen and Gordon M. Shepherd. Sections of Neurosurgery, Neuroanatomy and Anatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

A modified glomerular complex (MGC), situated at the dorsomedial junction of the main and accessory olfactory bulbs, exhibits increased functional activity during odor-dependent suckling in 10 day old rat pups. On morphological and functional grounds, this complex appears to be a specialized region of the olfactory system in rats of this age. Therefore, we have examined the structural development and functional status of this region during the neonatal period.

Rat pups 0,3,6,9,12 and 15 days old injected I.P. with ^{14}C-2-deoxyglucose (2DG) (200uCi/kg) and placed with their anesthetized dam where they suckled for one hour. The pups were sacrificed at the end of this period and their brains processed for autoradiography. At all ages foci of increased 2DG uptake were centered over the MGC. Prior to day 6 focal 2DG uptake was present only in the MGC, although diffuse regions of uptake were found elsewhere in the glomerular sheet of the main olfactory bulb. Histological examination of the olfactory bulbs of Bouin's fixed littermates demonstrated that the MGC was morphologically distinct from birth, unlike glomeruli of the main and accessory olfactory bulbs. These findings suggest that the MGC matures more rapidly than other regions of the olfactory bulb.

To examine the afferents to the MGC, the nares of older rats were injected with a solution of 20% horseradish peroxidase. Following a 48 hour survival, the rats were sacrificed and their olfactory bulbs removed and processed using the tetramethylbenzidine method. Dense reaction product was evident throughout the entire glomerular sheet of the main olfactory bulb and in the MGC. Only a few glomeruli in the accessory bulb were labelled. These findings strongly suggest that the MGC receives input from the main olfactory receptor sheet.

Further support for this finding has come from 2DG experiments in which partial ablation of the olfactory epithelium was performed using the $ZnSO_4$ method. Those rats exhibiting foci of 2DG uptake in the MGC, but not in the main glomerular sheet, had a small region of intact olfactory epithelium in the dorsal recess of the nasal cavity.

The results demonstrate that the MGC: 1) is present in rat pups of all ages from birth; 2) is functionally active at birth; and 3) receives some portion of its input from the main olfactory receptor sheet.

This work was supported by NIH Grant NS16993.

7.5 IN UTERO LOCALIZATION OF 2-DEOXYGLUCOSE IN RAT MAIN AND ACCESSORY OLFACTORY BULBS. P.E. Pedersen, W.B. Stewart, C.A. Greer, and G.M. Shepherd. Secs. of Neuroanatomy, Neurosurgery, and Gross Anatomy, Yale University School of Medicine, New Haven, CT 06510.

The vomeronasal system and the main olfactory system process odors concerned with sexual/social behaviors in adult rodent species. While the main olfactory system has been clearly demonstrated to be important throughout development, little is known about the functional importance of the vomeronasal system in neonates. This is despite the morphological maturity of receptors at birth and the early embryonic development of the accessory olfactory bulb, the first relay of the vomeronasal system. Further, postnatal behavior of rat pups is influenced by their prenatal experience with amniotic fluid, which suggests that one or both of these chemosensory systems may be functional in utero. In light of these considerations, we used the ^{14}C-2-deoxyglucose (2DG) method to assess functional activity in main and accessory olfactory bulbs of near term fetal rats.

Sprague-Dawley pregnant rats were injected with 200 µCi/kg 14C2DG I.V. on Day 22 of gestation. One hour later, the dam was killed by decapitation. Each fetus was immediately delivered by Caesarean section, its brain was removed and was prepared for autoradiographic analysis.

Enhanced 2DG uptake appeared in the accessory bulb of 18 of 20 experimental animals from 4 different litters. Correlations of autoradiographs with histological sections indicated that maximal 2DG uptake occurred throughout the entire glomerular sheet of the accessory bulb. Foci of 2DG uptake were evident in the main bulb of only 4 animals. These were localized in the dorsocaudal portion of the main bulb. This region usually shows 2DG uptake under odor stimulus conditions in very young postnatal rat pups. 2DG uptake also appeared to be prominent in the anterior olfactory nucleus; further analysis will indicate whether other central structures are involved.

These findings demonstrate that the 2DG technique can be used to investigate cerebral glucose metabolism in utero. Moreover, the high 2DG uptake in the accessory bulb suggests fetuses may use the vomeronasal system to sample and to learn about their chemical milieu prenatally.

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7.6 FLUORESCENT STUDIES OF THE PRIMARY OLFACTORY PATHWAY IN AMPHIBIA. Thomas V. Getchell. Department of Anatomy, Wayne State University School of Medicine, Detroit, MI 48201.

The cellular uptake and distribution of the fluorescent dye, Procion M-4RAN, has been studied in the olfactory mucosa of frogs and salamanders. The general techniques were modified from studies on fish olfactory epithelium (Holl, Stain Technology, 56:67, 1981) and monkey retina (Monasterio et al., Science, 213: 1278, 1981). The dye, 6% Procion/0.01M KCl, was delivered in three ways: a. intranasal irrigation, 0.5cc; b. submeningeal injection, 0.1cc and c. intravascular injection, 0.1cc. Following incubation for 40 min at 21°C and 1 hr at 4°C, the animals were perfused transcardially with fixative. The olfactory nasal sacs, nerves and bulbs were removed en bloc and prepared for fluorescent microscopy. As a result of intranasal irrigation, the dye was preferentially taken up by olfactory receptor neurons and to a much lesser extent by sustentacular cells, the cells of the ducts of Bowman's gland and cellular elements in the lamina propria. Typically, the entire receptor neuron was stained. The distribution of Procion-labeled neurons was not uniform throughout the sensory epithelium. But rather, assuming equal access of the dye to all "mature" receptor neurons, the dye was taken up by single neurons or small clusters of neurons. The reason for the apparent selectivity is not known, but may be related to the age of the receptor neuron, its metabolic state or functional activity at the time of exposure (Michael & Getchell, AChemS IV). As a result of submeningeal injection, small fascicles of fibers in the olfactory nerve layer of the olfactory bulb were preferentially labeled as well as large neurons deeper in the olfactory bulb. Retrograde labeling of receptor neurons in the olfactory epithelium was not seen. As a result of intracardial injection, the infranuclear region of sustentacular cells in the olfactory epithelium was labeled. Other studies (Rafols & Getchell) have shown that the basal expansion of this cell may terminate in close apposition to the capillary vasculature in the lamina propria. These initial results suggest that the use of Procion and other fluorescent dyes may be useful in labeling specific cells in the olfactory epithelium and in studying the cellular uptake and transport of molecules by the sustentacular cells from the blood vascular system.

7.7 ULTRASTRUCTURE OF SYNAPSES AND CILIA IN MOUSE VALLATE AND FOLIATE TASTE BUDS. J.C. Kinnamon, B. Taylor* and S. Roper. Anatomy Dept., Univ. of Colorado Med. Sch., Denver, CO 80262.

Using transmission electron microscopy (TEM) and high voltage electron microscopy (HVEM) of serial thin (<0.1 µm) and thick (0.5-1.0 µm) sections, we have characterized cell types and synapses found in mouse taste buds (TBs). With TEM and HVEM we have observed examples of Type I (dark), Type II (light), Type III and Type IV (basal) cells.

Cilia were found associated with Type I cells. These cilia contain microtubules which extend from an axial centriole. To date, however, we have not observed oblique centrioles, which are usually associated with cilia. The cilia do not appear to have a preferred orientation, and none have yet been found to extend into the taste pore. Hence we doubt that they play a primary role in gustation.

Synaptic contacts were found throughout vallate and foliate taste buds. In all instances observed to date the taste cell has been presynaptic and a neuronal process postsynaptic. We have tentatively classified these synapses based on their ultrastructural features and the density and quantity of synaptic vesicles. The type of synapse found with the highest incidence is characterized by a thickened presynaptic membrane containing periodic dense projections to which vesicles are closely apposed. A cleft of 16-30 nm separates the presynaptic region from the postsynaptic membrane. The postsynaptic densities are generally not as thick as the presynaptic thickenings. Vesicles in the presynaptic region are usually clear, but dense-cored vesicles may be present. These active zones are often characterized by gaps or breaks. Based on numerous observations of this phenomenon we speculate that these profiles may represent sections through an active zone, the shape of which is an annulus. We plan to analyze serial thin and thick sections to determine their exact shape. The second type of synapse has been observed much less frequently. It is characterized by a semicircular profile in which vesicles are clustered at the convex presynaptic density in higher numbers than are found in the first type of synapse. The presynaptic density is more uniform in the second type, but the periodic dense projections are less regular than those in the first type of synapse. In one instance we observed both types of synapses formed by a single taste cell onto what appeared to be separate neuronal processes.

The biological significance of our observation of two types of synapses is unknown, but we speculate that the differing synaptic structures may be indicative of functional differences. Future electrophysiological studies will be necessary to verify or disprove this hypothesis. This study was supported in part by NIH grant NS11505.

7.8 CAPSAICIN DESENSITIZATION OF TRIGEMINAL RECEPTORS TO ODORANTS. Wayne L. Silver[1,2] and Joel A. Maruniak[2]*. [1]Monell Chemical Senses Center; [2]Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Trigeminal receptors in the nasal cavity have long been known to respond to chemical stimuli. These free nerve endings make up part of the common chemical sense whose primary function is to elicit protective reflexes when stimulated with irritating compounds. However, the common chemical sense has also been shown to interact with olfaction to produce an overall odor sensation (Cain, 1974). An important question is whether these two functions are independent of each other, i.e., can stimuli which do not elicit painful or irritating sensations stimulate trigeminal receptors.

Repeated injections of capsaicin in rats desensitizes pain receptors to chemical stimuli (Jancso, 1960). We used this densensitization procedure and then recorded multiunit integrated responses from the ethmoid nerve to odor stimuli delivered via an air dilution olfactometer. No responses were obtained from capsaicin treated rats to amyl acetate or cyclohexanone, although tactile responses to touching the nose were just as vigorous as in controls. Propionic acid elicited responses at very high concentrations, > 987 ppm, in 3 of 6 experimental rats although the responses adapted more rapidly than in controls. The responses of the control rats produced by the three test stimuli were similar to those previously reported (Silver and Moulton, 1982). These results suggest that odorants which elicit responses from the common chemical sense do so by stimulating pain receptors and that chemical stimulation of nasal trigeminal receptors should produce painful or irritating sensations.

7.9 PARABRACHIAL GUSTATORY NEURONS ARE SENSITIVE TO STIMULUS TOXICITY. T.P.Collins*and T.R.Scott. Dep't. Psychology and Institute for Neuroscience, Univ. of Delaware, Newark, DE 19711.

Acceptance-rejection reflexes for gustatory stimuli are complete at or below the midbrain level in both rats and humans. Rejection is directly related to stimulus toxicity. This implies that there is a measure of stimulus toxicity in the brainstem afferent code for taste quality. We stimulated the tongue with an array of 16 chemicals having oral LD_{50}'s of 5 to 29,700 mg/kg in the rat, and recorded the responses of 41 single taste neurons in the parabrachial area (PBA) of the pons. We analyzed basic response characteristics (latencies, spike rates, time courses) for a 5-sec poststimulus period and determined the pattern of activity evoked by each stimulus across the neuronal sample. We calculated a correlation matrix showing the similarity between response patterns elicited by each possible stimulus pair. From these similarity coefficients we generated a 3-dimensional space, the axes of which should represent those stimulus characteristics to which the neurons were differentially sensitive. While two of the axes could not be identified with any obvious stimulus characteristics, the third correlated +0.71 ($p<.01$; N=16) with LD_{50}'s. This result demonstrates that stimulus toxicity, which activates rejection reflexes and which leads directly to the hedonic evaluation of a tastant, is a major determinant of a PBA neuron's response. At the least, this means that these neurons can activate acceptance-rejection reflexes and can transmit a toxicity measure to hypothalamus and amygdala, areas classically associated with hedonics and anatomically connected to PBA. It does not exclude the possibility that the hedonic appreciation of a chemical is determined by the activity of these brainstem cells, without the involvement of higher-order neurons.

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7.10 EFFECT OF CONCENTRATION ON THE TOPOGRAPHIC CODING OF ODORANT QUALITY. Alan Mackay-Sim and Paul Shaman*. Department of Psychology, Univ. of Wyoming, Laramie, WY 82071, and Department of Statistics, Univ. of Pennsylvania, Philadelphia, PA 19104.

When electro-olfactograms (EOGs) are recorded from many sites distributed over the surface of the olfactory epithelium of the salamander, the relative amplitudes of these EOGs can be used to draw a "topographic map" of responses across the epithelial surface. For a particular odorant such a response map shows a peak of highest responsivity, with EOG amplitudes decreasing in all directions away from this peak. Recently it was shown that odorants may elicit unique patterns of responses which may differ both in the location of the peak response and in the slope of the decrement in responses around the peak (Mackay-Sim, Shaman and Moulton, 1982). We proposed that the differing response maps were generated by the distribution of receptor cells with differing response spectra: cells with similar responses are located in similar regions of the epithelium. We concluded that the olfactory epithelium probably conveys a spatial code of odorant quality. If this is so, then not only must different odorants elicit different response patterns, but these patterns should not vary with the concentration of a given odorant. The aim of the present study was to test this proposal. At each of 12 sites on the ventral epithelium of the salamander we recorded at least 3 EOGs to each of 3 concentrations of an odorant. In this way maps of responses were generated for 3 odorants (pinene, amyl acetate, propanol) at 3 concentrations. Each odorant was tested in 4 animals. The odorants, delivered in 1S pulses from an air-dilution olfactometer, were presented through 3 stimulators, one for each concentration, attached to the electrode holder. This arrangement eliminates physical variables in odorant flow which can independently vary the EOG. The results show that the epithelial location of peak responsivity does not vary with concentration. For each odorant, an increase in concentration led to increases in EOG amplitudes over the whole epithelium. This resulted in a decrease in the rate of EOG decrement away from the peak of highest responsivity. We conclude that receptor cells of different response spectra are not mixed randomly throughout the olfactory epithelium. Cells of similar responsivity tend to be grouped together. Consequently, the location of greatest receptor responsivity could be a major factor in coding olfactory quality since different odorants elicit maximal responses from different epithelial locations which do not change with odorant concentration.

Reference: Mackay-Sim, A., P. Shaman and D.G. Moulton (1982) J. Neurophysiol. (in press)

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7.11 TASTE PREFERENCES AND NEURAL TASTE SENSITIVITY IN RABBIT. Robert J. Contreras, Edythe Bird*& Donald J. Weisz. Yale Univ., Dept. of Psychol., New Haven, CT 06520.

With few exceptions the rabbit is not ordinarily the preparation of choice for studies in taste. For example, we have found only two studies that have examined taste preferences (Carpenter, 1956; Ganchrow, 1979) and two older studies that have examined peripheral taste sensitivity in rabbit (Pfaffman, 1955; Beidler, Fishman & Hardiman, 1955). To provide more information on a potentially valuable animal preparation, we have further investigated the taste preferences (and aversions) and neural taste sensitivity of rabbit.

Five adult male rabbits were given a two-bottle preference test between water and various molar concentrations of sodium chloride (.003,.01,.03,.1,.3,.4,.5), potassium chloride (.01,.03, .1,.3), quinine hydrochloride (.00001,.0001,.001), hydrochloric acid (.0001,.001,.01), sucrose (.003,.01,.03,.1,.2,.3), and sodium saccharin (.003,.01,.03,.1). These rabbits showed normal increasing preferences for increasing concentrations of sucrose and increasing aversions to increasing concentrations of quinine hydrochloride and of hydrochloric acid as expected from similar data in rat and hamster. The rabbits exhibited, however, a weak preference for sodium chloride, being maximal at .03M with preference decreasing with both weaker and stronger concentrations. Sodium saccharin was rejected at all concentrations. The stimuli can be rank ordered in terms of decreasing preference as sucrose > potassium chloride > sodium chloride > hydrochloric acid > sodium saccharin > quinine hydrochloride.

In addition, we assessed neural taste sensitivity by recording the whole nerve response of the chorda tympani in several rabbits using the same stimuli of the preference study but with the addition of .1M and .3M lithium chloride. Notable in this data was the two-component response to sodium chloride which consisted of an immediate excitatory response followed by a tonic inhibitory response for the weaker concentrations (.003-.1M). In addition, low concentrations of sodium saccharin (.02,.1M) also elicited a tonic inhibitory response. Finally, potassium chloride elicited a stronger transient excitatory response than did sodium chloride from .03 to .3M concentrations.

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7.12 EFFECT OF THE DIURETIC AMILORIDE ON THE SENSE OF TASTE. S. S. Schiffman and Elaine M. Lockhead*. Dept. of Psychiatry, Duke Medical Center, Durham, NC 27710.

The diuretic amiloride has recently been found to reduce the flux substantially across dog lingual epithelium (DeSimone et al., Science, 1981, 214, 1039). In order to determine whether specific ion channels are involved in taste reception, the effect of amiloride on taste intensity was examined for 6 stimuli: NaCl, KCl, sucrose, Na_2SO_4, $CaCl_2$, and K_2SO_4. Two pieces of filter paper cut in the shape of half-tongues were placed on the dorsal tongue surface for a total of 5 minutes. One filter paper was impregnated with 5×10^{-4} M amiloride. The other was soaked in deionized water. Circles of filter paper with a diameter of 1/2 inch impregnated with the stimuli were placed at symmetrical areas on the two sides of the tongue. The side of the tongue adapted to amiloride was presented with one stimulus of constant molarity, i.e. .2M NaCl, .3M KCl, .8M sucrose, .3M Na_2SO_4, .25M $CaCl_2$, and .45M K_2SO_4. The concentrations required to match the concentrations on the amiloride-side were determined. The results indicate that amiloride reduces the perceived intensity of NaCl, Na_2SO_4, and sucrose but has no effect on KCl, K_2SO_4, and $CaCl_2$.

8.1 SEQUESTERING OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN CHROMAFFIN GRANULES: A POSSIBLE HIGH MOLECULAR WEIGHT PRECURSOR Illana Gozes, Daniel T. O'Connor* and Floyd E. Bloom. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute, and the San Diego Veterans Administration Medical Center, La Jolla, California.

Vasoactive intestinal polypeptide (VIP) has a wide variety of biological activities including vasodilation, regulation of energy metabolism and possible neurotransmitter function. The multiple actions of VIP are correlated with a widespread distribution in both endocrine and nerve cells. In search for mechanisms of regulation of VIP activity we investigated the modes of storage of the peptide in pheochromocytoma, a tumor known to contain copious amounts of the peptide.

Chromaffin granules, the norepinephrine storage granules of pheochromocytoma, were isolated from 5 human pheochromocytoma tumors. VIP immunoreactivity was detected in all of the granule preparations paralleling the synthetic VIP antibody binding curve over a range of serial dilutions. In addition, column chromatography revealed an immunoreactive peptide peak comigrating with VIP. However, a high molecular weight immunorective material was also detected on the column. This high molecular weight material was further characterized by $NaDodSO_4$ gel electrophoresis followed by electroblotting onto nitrocellulose paper and detection by anti-VIP antibodies and secondary antibody conjugated to horse radish peroxidase. A 70,000 M_r immunoreactive band was identified, in which reactivity with anti-VIP antibody was inhibited by VIP; this band did not cross react with non related antibodies.

In conclusion, VIP may be stored as a high molecular weight precursor, sequestered into chromaffin granules, cleaved and then the peptide may be released. Such a process offers multiple control points and may allow simultaneous or synchronic release of biologically active VIP and other neurotransmitters coexisting in the same storage vesicles.

The antibodies used in this study were kind gifts of Drs. R. Benoit and J. Fahrenkrug. The research was funded by Del E. Webb Foundation grants.

8.2 EVIDENCE FOR AN OPIOMELANOTROPIN ACETYLTRANSFERASE IN RAT PITUITARY NEUROINTERMEDIATE LOBE. M. C. Chappell*, Y. P. Loh and T. L. O'Donohue. (SPON: O. Floody). Lab. of Clin. Sci., NIMH, Lab. of Dev. Neurobiology, NICHHD and NIGMS, NIH, Bethesda, MD 20205.

In studies investigating the characteristics of the enzymatic acetylation of α-MSH, we found two distinct acetyltransferase enzymes in rat neurointermediate pituitary lobe. These acetyltransferases are distinguished by pH optima, subcellular distribution and sensitivity to magnesium and several solubilizing detergents. A general acetyltransferase, as characterized in the rat anterior pituitary lobe and rat lens, has a pH optima of 7.4 and is significantly inhibited by magnesium at 3mM concentrations. Subcellular fractionation of anterior and neurointermediate lobes revealed that this enzyme is primarily localized in the cytosol fraction. In contrast, the second enzyme has a pH optima of 6.0-6.6 and is severely inhibited by the detergents Triton X-100 (0.1%) and CHAPS (10mM). This enzyme is specifically localized in the secretory granules of the neurointermediate lobe. Comparative substrate studies reveal that this acetyltransferase is also capable of acetylating β-endorphin (1-31) and the characteristics of β-endorphin acetylation were quite similar to MSH acetylation in terms of pH optima, subcellular localization and detergent inhibition. Furthermore, acetylation of β-endorphin is dramatically inhibited by deacetylated α-MSH, suggesting that these peptides compete for the same acetyltransferase. These results indicate this single acetyltransferase is responsible for the acetylation of opioid and melanotropin peptides, and we have named this enzyme opiomelanotropin acetyltransferase (OMAT). Since the N-acetylated and deacetylated forms of α-MSH and β-endorphin express markedly different biological potencies, the enzymatic N-acetylation of these peptides may be an important regulatory process in modifying their biological activity.

8.3 IDENTIFICATION OF A BRAIN-PITUITARY PATHWAY FOR SECRETIN. C. G. Charlton*, T. L. O'Donohue, R. L. Miller*, G. E. Handelmann* and D. M. Jacobowitz (SPON: J. Haskins). Lab. of Clinical Science, NIMH, Bethesda, MD 20205 and Dept. of Pharmacology, Coll. of Med., Howard Univ., Washington, D.C. 20059.

Secretin, a 27 amino acid polypeptide, was previously thought to be located solely in the small intestine. Recently secretin-like immunoreactivity (SLI) has been identified and characterized in extracts of rat brain. The SLI showed retention characteristics similar to that of the rat duodenum and synthetic pig secretin on a high pressure liquid chromatographic reverse phase column. Highest concentrations of SLI were observed in the pituitary with moderate quantities in the hypothalamus, thalamus and the olfactory bulb.

Dissection of the pituitary gland revealed a 45 fold higher concentration of SLI in the neurointermediate lobe as compared with the anterior lobe. In pituitary stalk-transected rats the SLI of the neurointermediate lobe was significantly decreased, while the SLI of the anterior lobe remained unchanged. The source of the immunoreactive secretin is unknown, but highest secretin concentrations in the brain were measured in the paraventricular and supraoptic nuclei. These data suggest an anatomical similarity between secretin and vasopressin peptidergic systems. Also similar to vasopressin, the injection of synthetic pig secretin in anesthetized hydrated rats caused significant dose-related antidiuretic effects.

These data suggest anatomical and pharmacological similarities between secretin and vasopressin. Whether there is any functional physiological interaction between the two systems remains to be determined.

8.4 THE SEQUENCE OF TELEOST GONADOTROPIN RELEASING HORMONE (GnRH): MULTIPLE FORMS OF GnRH IN THE ANIMAL KINGDOM, N. M. Sherwood, L. Eiden*, M. J. Brownstein*, Unit on Neuroendocrinology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20205, J. Spiess*, J. Rivier* and W. Vale, Peptide Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037.

GnRH-like material has been extracted from whole brains of salmon (Oncorhunchus keta). The immunoreactive GnRH was purified by molecular sieving on a Sephadex G-25 column and by reverse phase high pressure liquid chromatography (HPLC). The fish GnRH compared with mammalian GnRH is more hydrophobic as shown by HPLC analysis. In addition the two forms of GnRH are immunologically distinct in their reactivity with several antibodies. The fish GnRH may be widely distributed among different species; it is chromatographically and immunologically similar to the GnRH-like neurotransmitter found in the frog sympathetic ganglia. The amino acid composition and sequence of salmon GnRH shows that it differs from the mammalian decapeptide in two positions.

8.5 IN VIVO BIOSYNTHESIS AND TRANSPORT OF THE PEPTIDE NEUROTRANSMITTER CANDIDATE SUBSTANCE P IN THE STRIATONIGRAL TRACT OF INDIVIDUAL FREE-RUNNING RATS. J.E. Krause, J.P. Advis*and J.F. McKelvy. Dept. of Neurobiology and Behavior, State University of New York at Stony Brook, NY 11794.

The regulation of neuropeptide biosynthesis in the central nervous system (CNS) is an important aspect of the functioning of neuronal networks. We have approached this question by using as a model the striatonigral substance P (S) projection. Previous anatomical evidence suggested that SP neuronal perikarya in the basal ganglia (rostral corpus striatum) project ipsilaterally in a descending fashion to terminal fields within the substantia nigra. In the present studies, we have developed chemical methods for the purification of undecapeptide SP along the trajectory of this peptidergic system and have used them to assess in vivo biosynthesis and transport of striatonigral SP.

Rats were unilaterally cannulated (two 28 g cannulae) in the rostral corpus striatum (A: 1.5mm, L: 2.0 and 3.0mm, V: 5.0mm with respect to Bregma). The following day, either ^{35}S-methionine or an equimolar mix of ^{3}H-leucine and ^{3}H-proline was constantly infused into the striatum using an Alzet osmotic minipump delivery system at a rate of 1 ul/hr (750 uCi/rat/16 hrs). Under these in vivo labeling conditions, animals did not appear to be adversely affected since they exhibited normal motor and grooming behavior and/or slept during most of the infusion period. After decapitation, the ipsilateral corpus striatum (cell bodies), its striatonigral fasciculus (axons) and the ipsilateral substantia nigra (terminals) were separately microdissected from frozen coronal brain sections. Tissue samples were homogenized and ^{35}S- or ^{3}H-SP was acid extracted after carrier peptide addition. All individual samples were analyzed for total tissue ^{35}S or ^{3}H, 10% (w/v) trichloroacetic acid precipitable ^{35}S- or ^{3}H-protein and acid soluble ^{35}S or ^{3}H. ^{35}S- or ^{3}H- SP was purified from the latter fraction by adsorption to and elution from C-18 SEP-PAK cartridge, followed by sequential purification by High Performance Liquid Chromatography (HPLC). In all cases, the radiochemical purity of ^{35}S- or ^{3}H- SP was verified by specific derivative formation (Met^{11} sulfoxide-SP) and further HPLC.

In the corpus striatum, incorporation of ^{35}S or ^{3}H into tissue protein was greater than 70% of the total ^{35}S or ^{3}H taken up by the tissue. 35S- and ^{3}H-SP was positively identified in all three regions of this peptidergic pathway. Under these infusion conditions, radiolabeled SP was 20 to 80 fold more concentrated in the substantia nigra compared to the striatal and striatonigral fiber regions.

These results provide definitive biochemical evidence for the in vivo biosynthesis and transport of SP along this peptidergic striatonigral projection. (NSF BNS-7684506)

8.6 A PUTATIVE ENDOGENOUS PEPTIDE LIGAND FOR THE PHENCYCLIDINE RECEPTOR. C.B. Pert, T.L. O'Donohue, H. Everist, A. Pert and R. Quirion. Neuroscience Branch and Lab. of Clin. Sci., NIMH, Bethesda, MD 20205.

Specific [^{3}H]phencyclidine (PCP) binding sites on slices of rat brain can be displaced by a series of PCP analogs with the identical ($p < .001$) ligand selectivity pattern as that required to elicit discrimination of a PCP-like state in an awake behaving rat (Quirion et al., PNAS, 78:5881, 1981). The regional distribution of these specific PCP receptors has been visualized by computer-assisted densitometry (Quirion et al., ibid) and the relative receptor density is hippocampus > cerebellum > striatum > brainstem. The possibility that the brain contains an endogenous ligand for this receptor is supported by our finding that an efficient peptide extraction procedure (Bennett et al., Biochem. J., 175:1139, 1978) yields [^{3}H]PCP receptor inhibitory material with a regional distribution pattern similar to the receptors themselves (i.e., hippocampus: cerebullum: striatum: brainstem = 12: 3: 2: 1). Moreover, a high presssure liquid chromatographic (HPLC) fraction purified from porcine hippocampus selectively displaces [^{3}H]PCP binding in a dose-dependent manner. The same fraction in a concentration which displaces [^{3}H]PCP binding more than 70% fails to displace the binding of [^{3}H]dihydromorphine, [^{3}H]D-Ala^{2}, D-Leu^{5}enkephalin, [^{3}H]ethylketocyclazocine, [^{3}H]diazepam or [^{3}H]neurotensin. The ability of the fraction to inhibit [^{3}H]PCP binding is destroyed by preincubation with various enzymes including pronase, trypsin, leucine-aminopeptidase and carboxypeptidase A, suggesting that this endogenous inhibitor is a peptide. Finally, unilateral injection of this active material into rat substantia nigra induces PCP-like rotational behavior. Injection of an adjacent HPLC fraction with no PCP-inhibitory activity is without effect. These results spur us to determine the chemical structure of a putative endogenous peptide ligand for the phencyclidine receptor which we call "angeldustin."

8.7 EXTRAHYPOTHALAMIC VASOPRESSIN: IMMUNOLOGIC AND CHROMATOGRAPHIC BEHAVIOR, AND ITS REGULATION. D.M. Dorsa* and L.A. Bottemiller* (SPON: L. Halpern). GRECC, VA Medical Center, and Depts. of Pharmacology and Medicine, Univ. of Washington, Seattle, WA 98108.

Extrahypothalamic brain vasopressin (VP) may play an important role in CNS thermoregulatory, cardiovascular, and behavioral functions. In the present study, we examined the chemical nature of VP-immunoreactive (VP-IR) peptides inside and outside the hypothalamus and the effect of water restriction (WR) on VP content in selected brain nuclei.

For regulation studies, male Long-Evans rats were water deprived for 48 h (n=10) or given water ad lib (n=10) and were sacrificed by decapitation. Plasma was collected, and the brains and pituitaries were rapidly removed and frozen. Brain nuclei were sampled by a punch technique. Plasma VP was measured in an RIA after extraction using a Sep-Pak C_{18} procedure. Tissue VP was extracted by sonication in 0.1 N HCl, centrifugation, and lyophilization and was then reconstituted for VP RIA.

Ten other rat brains were removed, and the hypothalami were dissected out. 10% (w/v) homogenates of the hypothalami and remaining brain were prepared using 0.1 N HCl. Homogenates were centrifuged at low speed. Supernatant was lyophilized, reconstituted, and passed through a Sep-Pak C_{18} column and eluted in acid-ethanol. The concentrated samples were then fractionated using a phosphate buffer-acetonitile gradient HPLC system. 0.5 ml fractions were collected, air dried, and reconstituted for measurement of VP-IR.

VP content of the posterior pituitary decreased and plasma concentration increased significantly in the WR group. VP content of the magnocellular nuclei was not significantly altered by this treatment, nor was that of the OVLT, locus coeruleus, dorsal septum, or central grey. Outside the hypothalamus, lower VP content was measured in the ventral tegmentum and lateral and medial septum of WR rats. An increase was noted in samples from the medial amygdala. These responses to WR suggest that some VP-ergic neurons are not affected by this procedure.

In the radioimmunoassay, IR-VP extracted from hypothalamus, septum, and hippocampus was immunologically identical to the USP standard or synthetic arginine^{8}-vasopressin (AVP). The HPLC method separates AVP from oxytocin (OXY), arginine-vasotocin (AVT), pressin amide (PA), and proly-leucyl-glycinamide (PLG). Hypothalamic extract was resolved as a single peak of immunoreactive material which co-eluted with AVP. Extractant from extra hypothalamic brain tissue also contained primarily AVP co-elutable material, but in addition, a smaller peak of VP-IR was noted which did not co-elute with OXY, AVT, PA, or PLG. This VP-IR material may reflect another secretory product of certain nerve terminals or metabolism of synaptically released AVP.

8.8 BIOCHEMICAL CHARACTERIZATION OF RETINAL SOMATOSTATINS: SEQUENCE OF BIG SOMATOSTATIN IN BOVINE RETINA. D. Marshak*, J. Reeve*, J. Shively*, and T. Yamada* (Spon: G. Fain). Depts. of Anatomy and Medicine, UCLA School of Medicine and VA Wadsworth Medical Center, Los Angeles, CA 90073.

Previously we have identified somatostatin-like immunoreactivity (SLI) in a variety of vertebrate retinas and localized it to amacrine cells (PNAS 77:1691, 1980). In the present study we further characterized the biochemistry of retinal SLI. Extracts (3% acetic acid) of retinas from goldfish, frogs, chickens, rats, hogs, and cows were chromatographed Sephadex G50 in 0.1M ammonium acetate pH5.0 and eluted fractions were measured for SLI by radioimmunoassay. All of the extracts, with the exceptions of those from rat and cow retinas exhibited roughly equal distribution of SLI into peaks which co-eluted with somatostatin-14 (S14) and -28 (S28). Rat retinal SLI chromatographed in a single peak which co-eluted with S14. Bovine retina exhibited a major peak (>90%) which co-eluted with S28 and a minor peak which eluted in the column void. Re-chromatography of the bovine retinal extract in 8M urea resulted in disappearance of the void volume peak and appearance of a peak co-eluting with S14, suggesting that the latter was non-covalently attached to a larger molecule in the crude extract. Further purification for sequence analysis was practical only with bovine retinal SLI. Thus, extracts of 3000 bovine retinas were brought to pH5.0 with ammonium hydroxide and applied to an affinity column made by linking anti-S14 antiserum 1001 to Affi-Gel 10. The SLI which was eluted with 2% trifluoroacetic acid (TFA) was applied to reverse phase high pressure liquid chromatography (HPLC) on a C-18 column and eluted with a gradient of 0-30% acetonitrile in 0.1% TFA. The major SLI peak was further purified by HPLC twice on a phenyl column using a gradient of 25-30% acetonitrile in 0.1% TFA until a single clean A_{220} peak was found to correspond to the peak of SLI. The peptide sequence of this peak as determined by an automatic Edman degradation technique performed with a modified spinning cup sequenator was SANSNPAMAPRERKAG(C)KNFFWKTF(T)(S)(C), identical to S28. The amino acids in parentheses could not be determined directly but were deduced because 1) the purified peptide co-eluted with S28 on HPLC, 2) the purified peptide had complete immunoreactivity while immunoreactivity of peptides substituted in positions 17, 26, 27, and 28 was virtually abolished, and 3) all previously sequenced mammalian big somatostatins have this identical sequence.

We conclude that 1) SLI is present in 2 molecular forms in a variety of vertebrate retinas, 2) rat retina contains only one form of SLI which co-elutes with S14 on gel filtration, and 3) the sequence of big SLI in bovine retina appears to be identical to that of S28.

8.9 NON-MAMMALIAN LUTEINIZING HORMONE-RELEASING FACTOR (LRF) IN TADPOLE AND FROG BRAIN. W. Dale Branton*, L.Y. Jan and Y.N. Jan. Department of Physiology, University of California, San Francisco, CA 94143.

Adult bullfrog brain contains a large amount (approximately 25 ng) of mammalian LRF. Bullfrog sympathetic ganglia contain about 0.2-1.0 ng of an LRF-like neurotransmitter distinct from mammalian LRF. We have now found a second brain immunoreactive LRF (LRF II) which may be similar to the ganglionic LRF.

Brain LRF II is present at about 0.1-0.4 ng per animal from metamorphic tadpoles through adult frogs. It makes up most or all of the LRF immunoreactivity in tadpoles, but mammalian LRF predominates in post-metamorphic frogs and greatly predominates in the mature adult where the non-mammalian form is probably at most 1-2% of the total brain LRF.

The brain LRF II is clearly separable from mammalian LRF on reversed phase HPLC and adsorption chromatography on G-25 Sephadex. It also differs in the slope of its RIA dilution curve (Nett R-42 antiserum). On the other hand, we have not found any differences between the chromatographic or immunologic behavior of the non-mammalian brain LRF II (tadpole source) and the ganglionic LRF. This suggests a relative similarity between these peptides. It is interesting to note the difference in the regulation of amounts of mammalian vs. non-mammalian forms of LRF in the frog brain.

8.10 ISOLATION OF NOVEL, NEUROACTIVE, ELH-LIKE PEPTIDES FROM THE ATRIAL GLAND OF APLYSIA. B.S. Rothman†, R.O. Brown*†, E. Mayeri†¶, and J.E. Shively*§. †Dept. of Physiology, Univ. of Calif., San Francisco, CA 94143, ¶Dept. of Basic Sciences, Calif. College of Podiatric Medicine, San Francisco, CA 94115 and §Div. of Immunology, City of Hope, Duarte, CA 91010.

Egg-laying hormone (ELH) is a peptide synthesized by the bag cell neurons of Aplysia that very likely mediates two bag cell actions on neurons of the abdominal ganglion. Three peptides isolated by others from the atrial gland of Aplysia cause the bag cells to fire: peptides A & B, which are unrelated to ELH in amino acid sequence and a hybrid peptide which has a strong sequence homology to the N-terminal half of ELH and the C-terminal halves of peptides A and B. We have purified extracts of the atrial gland to explore further the neuroactive peptides in this tissue.

Atrial glands were homogenized in 0.5 M formic acid at 0°C and fractionated on G50 Sephadex in 0.5 M formic acid. Material with an apparant M_r of 3000-10,000 was lyophylized, applied to a Partisil 10-ODS high performance liquid chromatography (HPLC) column and fractionated with a linear gradient of n-propanol in 0.5 M pyridine formate, pH 3.0. Among the major peaks of eluted material, the following five peptides were identified (in order of elution): peptide B, peptide A, and three ELH-like peptides called C, D and E. The yield of each peptide per atrial gland was (mg): 0.70, 0.67, 0.32, 0.14 and 0.09, respectively. Peptides D and E were not completely separated by the first HPLC step and were repurified on Partisil 10-ODS under isocratic conditions.

Peptides A & B were identified by amino acid and sequence analyses. Peptides C, D and E cross reacted strongly in an enzyme linked immunosorbant assay with affinity purified rabbit antibodies to ELH, while peptides A & B did not cross react. Peptides C, D & E, but not A or B, caused the prolonged excitatory response characteristic of left lower quadrant cells exposed to ELH. The thresholds for the responses to peptides C, D & E ranged from 0.5 to 5.0 ug/ml, while the threshold for ELH was 0.1 ug/ml. Amino acid and partial sequence analyses indicated that the N-terminal halves of peptides C, D & E were identical or strongly homologous to the N-terminal sequence of ELH. The analyses also suggested that neither peptides C, D nor E is identical to the hybrid peptide. Furthermore, all three peptides eluted differently from ELH under isocratic conditions on HPLC.

These data show that there is a family of ELH-like peptides in the atrial gland distinct from the ELH found in the bag cells. In addition, they imply that the N-terminal halves of ELH and peptides C, D & E are responsible for their prolonged excitatory activity in the CNS.

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8.11 PITUITARY CHOLECYSTOKININ (CCK)/GASTRIN PEPTIDES: CHARACTERIZATION BY HPLC AND RIA. M.C. Beinfeld* (SPON: J. Taylor). Dept. of Pharmacology, St. Louis Univ. Med. Ctr., St. Louis, MO 63104.

CCK/gastrin immunoreactive pituitary peptides were characterized by radioimmunoassay, gel filtration, and high pressure liquid chromatography (HPLC). The concentration of CCK-like immunoreactivity (CLI) was measured in whole and dissected pituitaries with a CCK radioimmunoassay (RIA) which cross-reacts 80% with human gastrin I. Whole human pituitaries contained the highest concentration of CLI: 518.7 ± 166.5 ng/g wet weight (N = 6). The neurointermediate (NI) lobes and the pituitary stalk (PS) from bovine and porcine pituitary contained the most CLI relative to the anterior lobe [bovine NI: 439.2 ± 86.8 (N = 7), bovine PS 107.4 ± 27.5 (N = 5); porcine NI: 14.4 ± 1.7 (N = 7) and porcine PS: 21.7 ± 2.7 (N = 6)]. In rat and frog pituitary the NI or neural lobe also contained the highest CLI concentration. This distribution is suggestive of a possible hypothalamic origin of the CLI in these species. In rat we have demonstrated that 60% of the neural lobe CLI originates in the paraventricular nucleus of the hypothalamus and that all of the CLI in the pituitary originates in the brain (Beinfeld et al. (1980), Nature 288:376-378).

Whole bovine and human pituitary gland extracts display a single peak of CLI on Sephadex G50 which co-eluted with CCK8 sulfate. Most of the CLI in extracts from bovine anterior lobe, neurointermediate lobe or pituitary stalk also co-migrated with CCK8 sulfate on HPLC. The observation that the bulk of CLI in bovine pituitary co-migrates with CCK8 sulfate differs from the results of Rehfeld (1980), Nature 271:771-778. Preliminary results with whole human pituitary extracts also indicate that the bulk of the CLI in these extracts co-elutes with CCK8 sulfate on HPLC.

In contrast, the CLI in porcine pituitary extracts eluted as two peaks on Sephadex G50, one which co-migrated with Gastrin 17 and one that precedes it, while no CCK was present. This result is in agreement with the work of Rehfeld ((1980), Nature 271: 771-778). On HPLC the bulk of the CLI in porcine pituitary extracts eluted with porcine antral gastrins, and separates from CCK8.

In summary, clear species differences exist in the CCK/gastrin pituitary peptides. This points out the necessity of careful chemical characterization of pituitary gastrin/CCK peptides prior to physiological or pharmacological experimentation.

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8.12 POLYPEPTIDES IN LUNG: CONTENT AND SITE OF ACTION. J. Tang*, P. Panula*, J. Chou*, A.Z. Zhang, H.-Y.T. Yang and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

The lung contains many APUD-like cells, but the function of these cells is still unknown. With specific and sensitive radioimmunoassay coupled with gel filtration, HPLC and immunofluorescence we explored the presence and measured the content of met^5-enkephalin, β-endorphin, met^5-enkephalin-arg^6-phe^7 (YGGFMRF), bombesin and substance P in the lung. YGGFMRF content in lungs of rat, guinea pig and human was found to be 0.68±0.08, 0.76±0.12 and 0.63±0.14 pmol/mg protein, respectively. Histochemical studies detected cells exhibiting YGGFMRF-like immunoreactivity in lung; they were located in clusters or as single cells closely associated with the wall of small and medium size bronchioli. This immunostaining was diminished when the antiserum was preabsorbed with YGGFMRF but not with met^5-enkephalin. Biochemically little or no met^5-enkephalin (ME) and β-endorphin was detected in lung.

Using ^{3}H-etorphine binding assay we observed high affinity (K_D=5.0) binding (Bmax 40 pmol/mg protein) in rat lung membrane preparations. And this binding could be displaced by YGGFMRF with high affinity. However the recognition site for opiates located in rat lung membranes shows a number of peculiarities including a low affinity for ^{3}H-naloxone.

Bombesin and substance P were also measured in lung of rat and humans. Histochemically both bombesin and substance P were located in axons. The bombesin and substance P contents in rat lung were 0.94±0.14 and 1.9±0.06 pmol/mg protein. These data allow us to speculate that YGGFMRF may be released from APUD like cells and acts on axons to elicit important respiratory changes (J pulmonary receptor) in rate and depth of respiration. The stimuli that release YGGFMRF are not known at this time.

9.1 DEVELOPMENT OF SENSORY NERVES WITHIN THE WING OF DROSOPHILA MELANOGASTER: A LIGHT- AND ELECTRON- MICROSCOPE STUDY. H. Anderson. European Molecular Biology Laboratory, 6900 Heidelberg, West Germany.

The Drosophila wing is a simple structure bearing many sensory neurons which form an elementary pattern of nerves within the veins. This simplicity and the availability of many genetic mutants make the wing an attractive system for studying the formation of nerve pathways.

The wing develops during the pupal stage of fly development. A light- and electron- microscope study of developing wings at timed pupal stages was undertaken first to establish the time at which the different classes of sensilla differentiate and form axons, and second to determine the arrangement of tissues within the wing prior to, and during, axon outgrowth, in order to assess possible candidates for directing axon outgrowth.

Wing development was observed to pass through three main stages: at 6-12 hours after puparium formation the wing is flattened and secreting pupal cuticle; at 15-18 hours the wing is dramatically inflated but is still a simple epithelium secreting pupal cuticle; at 21-24 hours the wing collapses and begins to secrete adult cuticle and to differentiate adult structures.

Each bristle on the anterior margin of the wing and each campaniform sensillum on the blade of the wing is made from a group of cells (usually a shaft cell, a socket cell, one or more neuron sheath cells, and one or more sensory neurons) derived from the mitotic divisions of a single epithelial cell. Differentiation of the sensory neurons begins before differentiation of the other cells; axon bundles are first detected during the 15-18 hour stage prior to the pupal moult when the wing is still secreting pupal cuticle, but morphological differentiation of the shaft and socket cells does not begin until the 21-24 hour stage which is after the pupal moult when the wing is secreting adult cuticle.

Even at the earliest stages of development there appear to be no persisting larval nerves which might act as pioneers or pathfinders for the later developing neurons. The first axon bundles are detected at the stage when the wing is an inflated sac, without any veins or orderly arrangements of tracheae or obvious physical regularities which might mechanically channel axon growth. Rather axons appear to navigate along the inner surface of the wing epithelium rather like the pioneer axons found in the embryonic appendages of other insects.

9.2 IMMUNOCYTOCHEMICAL STUDIES OF NEURAL DEVELOPMENT IN DROSOPHILA. Efrain Aceves-Pina*, Sandra Barbel*, Louise Evans*, Yuh Nung Jan and Lily Yeh Jan (SPON: Cheryl Laffer Shusterman). Department of Physiology, University of California, San Francisco, CA 94143.

Monoclonal antibodies have been used extensively in developmental studies of vertebrates and invertebrates. Drosophila may may be a favorable organism for such studies because of its well-studied genetics and molecular biology. Here we describe some monoclonal antibodies that recognize various structures of the nervous system at different developmental stages. These monoclonal antibodies were induced by either injecting and boosting mice with Drosophila embryos at various developmental stages (0-4 hr, when neuroblasts appear; 7-10 hr, when central fiber pathways form; or 18-22 hr, the final stage of neural organization; in each of 3 fusions), or injecting mice with 7-10 hr Drosophila embryos and boosting with the grasshopper embryonic nervous system at a similar developmental stage (done in collaboration with Katherine Kotrla and Corey Goodman at Stanford University).

Embryogenesis of the nervous system in Drosophila has been studied using monoclonal antibodies which recognize neuronal nuclei or subsets of nerve fibers early during embryonic development. Further, an interesting developmental profile has been found for certain antigens. For instance, some monoclonal antibodies stained all nuclei in young embryos but preferentially neuronal nuclei plus a subset of nerve fibers later during development.

Although these monoclonal antibodies were raised against embryonic tissues, some serve to subdivide the visual system in adult flies. For instance, some antibodies preferentially stain (1) retina, (2) lamina and/or medulla, (3) cells in lobula and lobula plate, or (4) subsets of fibers in all optic ganglia. Immunocytochemical studies of visual mutants and mutations affecting early neural development in embryos will be described.

9.3 THE LABELING PATTERNS OF DEVELOPING LEECH CNS NEURONS WITH MONOCLONAL ANTIBODIES RAISED AGAINST ADULT NERVOUS TISSUE. R.R. Stewart[1], E.R. Macagno[1], and B. Zipser[2]. Columbia University[1], New York, NY 10027 and Cold Spring Harbor Lab[2], Cold Spring Harbor, New York, NY 11724.

Among the monoclonal antibodies (Mabs) raised against the adult nervous system of the leech Haemopis marmorata by Zipser and McKay (Nature 289:549-554, 1981), some label groups of neurons in each segmental ganglion, indicating that the same or a very similar antigen is expressed by different adult neurons. In the experiments we report here we have studied the pattern of labeling of CNS neurons at different development stages by two of these Mabs (Lan 3-1 and Lan 3-6). Our aims were, first, to describe the temporal sequence of expression of the antigens to these Mabs, and second, to determine how the development of segmental differences (particularly in the sex ganglia of body segments 5 and 6) correlate with the expression of segmentally different patterns of labeling with Mabs. In the adult, Lan 3-6 interacts with about 35 neurons (35±9, n=26 ganglia) in each segmental ganglion. Lan 3-1, however, labels only one pair of small neurons in each segmental ganglion except for 5 and 6, where it labels an additional, larger pair per ganglion. We used the same techniques to visualize antibody binding in embryos as were used by Zipser and McKay to study adults.

Embryogenesis in H. marmorata lasts about a month at 22-25°C. The first ganglia to form are observed at 4-5 days of development, and all the ganglia can be visualized, adjacent to one another, by 8-9 days. Within the next 2-3 days the first four ganglia fuse to form the subesophageal ganglion, the midbody ganglia separate as the connectives grow in length, and the posterior seven ganglia fuse to form the tail ganglion. Labeling with Lan 3-6 is first observed at 8-9 days, when a pair of neurons located postero-medially on the ventral side of each of the most anterior segmental ganglia is labeled. As a function of time after this stage we observe, first, that more neurons label in the anterior segmental ganglia, and second, that labeled neurons appear in increasingly more posterior ganglia. For instance, in an 8-9 day old embryo, ganglia 4-7, 10-13 and 17-20 show, on the average, 2, 1 and 0 labeled cells respectively. In an 11-12 day old embryo, the same ganglia show $6 \pm 1, 4 \pm 2$, and 2 ± 1 labeled cells. In a 12-13 days old embryo the corresponding values are 13 ± 5, 10 ± 1 and 6 ± 2. Labeling with Lan 3-1 is first observed at about 12 days of development, when all segmental ganglia show a pair of labeled neurons located postero-laterally on the ventral side. The additional pair of neurons in ganglia 5 and 6 that label in the adult do not label in 12 day embryos, nor in embryos up to 15 days old. Whether this delay in the labeling of cells specific to the sex ganglia correlates with our preliminary observation that the maturation of these ganglia is also delayed remains to be confirmed.

9.4 ANTIBODY STAINING OF EMBRYONIC LEECH MUSCLE, BLAST CELL MIGRATION AND NEURONAL PATHWAY FORMATION. Duncan K. Stuart, Ian Thompson*, David A. Weisblat and Andrew P. Kramer. Dept. of Molecular Biology, University of California, Berkeley, CA 94720.

Early differentiating muscle cells are located along pathways of embryonic blast cell migration and nerve tracts and, therefore may be important for guidance of cell migration and axonal growth. This suggestion has been investigated by staining the muscles in disected embryos of the leeches Helobdella triserialis and Haementeria ghilianii with the monoclonal antibody Lan 3-14 of Zipser and McKay (Nature 289, 549, 1981) which they have shown to react specifically with adult leech muscle.

The antibody does not stain blast cell precursors but only differentiated muscles. The first cells to stain in the germinal plate appear at stage 8 of development. There is one bilateral pair of such cells per segment. Oblong in shape, they extend transversely from the ventral midline to the lateral edge of the germinal plate. They correspond to circular muscles and define the border between segments. The next stainable cells appear at early stage 9; they form two bilaterally paired sets of about ten longitudinal muscle cells per segment, each cell extending from the circular cell defining the anterior border of the segment to that defining the posterior border. The positions of these longitudinal muscle cells are not evenly spaced across the segment. At about this time two pairs of cells which will become the longitudinal muscles of the connectives and a second pair of circular muscles (parallel to the first) also start to stain. This second set of circular cells are located two thirds of the way back from the anterior edge of the segment. Other longitudinal and circular muscles appear later in stage 9 and are initially thinner and less intensely staining. Subsequently, still more circular and longitudinal muscle cells appear adjacent to the preexisting ones so as to form specific muscle bundles. Oblique muscles develop later, in early stage 11.

The antibody stain has been used on embryos in which certain blast cells have been previously labeled with a cell lineage tracer (Weisblat et al, Science 209, 1538, 1980) or a tracer injected into developing, identified neurons. Specific muscle cells of a given type differentiate at different times. The earliest differentiating cells are spatially associated with pathways of blast cell migration and peripheral axon growth.

9.5 MUSCLE CELLS, GLIAL CELLS AND AXONAL GUIDANCE IN LEECH EMBRYOS. Andrew P. Kramer and Duncan K. Stuart. Dept. Molecular Biology, Univ. Calif., Berkeley, CA 94720.

Growing axons of central neurons in leech embryos may be guided initially by muscle or glial cells in the CNS and periphery. Every major axon tract in the leech nervous system develops in close spatial association with specific muscle or glial cells. First to form are the commissural tracts. The initial processes of central neurons grow toward the center of the ganglion, which is dominated by two giant neuropil glial cells aligned on the midline. The first commissural axons grow just dorsal to or exactly between the glial cells. Subsequently, axons grow between the ganglia to form the connective tracts. Before axonal outgrowth, the space between adjacent ganglia is bridged only by two pairs of connective muscle cells dorsally and a pair of connective glial cells ventrally. The first connective axons grow between the muscle and glial cells. Some follow the lateral connective muscle cells, to form the paired lateral connective nerves, and the rest follow the medial muscle cells, to form the unpaired Faivre's nerve. Finally, axons grow into the periphery, which at this stage is occupied by an orthogonal grid of longitudinal and circular muscles and also dorso-ventral flattener muscles. The first peripheral axons grow along these muscles, and identified axons predictably follow particular muscle pathways. This was ascertained by combining selective staining of muscle cells using an antibody (Lan 3-14; Zipser and McKay, Nature 289:549, 1981) and intracellular dye-filling of identified neurons that have peripheral axons. In these preparations, one can see a close spatial association between growing peripheral axons and muscle cells. This is true not only for axons of motor neurons but also for peripheral axons of central sensory neurons that innervate the skin. The dorsal (P_D) and ventral (P_V) pressure-sensitive mechanosensory neurons that appear to pioneer the MA and DP peripheral nerve tracts (Kuwada and Kramer, in press) reach their appropriate skin territory by growing along particular muscle cells before entering the epidermis. The P_D neuron, which innervates dorsal skin, grows along the medial bundle of flattener muscles, that is a bridge over ventral skin to dorsal skin territory. The P_V neuron grows along the grid of circular and longitudinal muscles in the ventral territory. Its axon initially grows laterally along circular muscles in the middle of the body segment and extends longitudinal branches as it passes over each of three specific longitudinal muscle cells. Each of these branches in turn extends lateral branches when it reaches circular muscles at the sides of the body segment. We suggest that the muscle cells at these branch points may be landmarks recognized by the P_V axon.

9.6 MONOAMINE-CONTAINING NEURONS OF THE LEECH AND THEIR TELOBLAST OF ORIGIN. S.S. Blair and D.K. Stuart. Dept. Molecular Biology, Univ. California, Berkeley, CA 94720.

The injection of tracer dyes into specific, identifiable blastomeres (teloblasts) in the early leech embryo leads to the reproducible staining of particular portions of the embryonic germinal plate, including the nervous system. Therefore, it has been proposed that specific neurons arrise only from the progeny of a particular teloblast. To test this hypothesis two types of studies have been performed, using either the ablation of identified teloblasts or the injection of dyes to trace cell lineages.

The monoamine-containing neurons of the leech CNS and body wall may be detected by using the glyoxylic acid technique. In this way each segment has been shown to contain on one side three serotonin-containing cells in the CNS and three dopamine-containing cells in the body wall. In the first type of study, adults have been reared from embryos in which particular teloblasts were ablated through the injection of DNase I. Adults in which one N teloblast was deleted lack those serotonin-containing neurons. Adults lacking one OP cell lack the two more lateral dopamine-containing neurons. Adults lacking the Q teloblast lack the medial dopamine-containing neurons. For the second type of study, glyoxylic acid staining was used on embryos in which selected teloblasts were injected with a rhodamine-labeled peptide. In this way it has been determined that, in agreement with the above results, the Q teloblast gives rise to the medial dopamine cell, the O teloblast to one lateral dopamine cell (LD2) and the P teloblast to the other (LD1). Work is currently underway to directly determine the teloblast from which the serotonin-containing neurons are derived by using antiserum against serotonin to identify these neurons in embryos similarly injected with tracer dyes.

9.7 DEVELOPMENT OF SWIMMING IN THE LEECH FOLLOWING ABLATION OF SEROTONIN-CONTAINING NEURONS. J.C. Glover* (Spon: W.B. Kristan, Jr.). Dept. of Biology, UCSD, La Jolla, CA. 92093

Leech embryos were treated with 5,7-dihydroxytryptamine, causing a selective and complete ablation of all serotonin-containing neurons. The embryos continued to develop and were examined for behavioral abnormalities at later stages. Particular attention was paid to swimming behavior, which is known to be stimulated by serotonin. Swimming behavior develops in a sequence of stages involving gradual refinement of more primitive movements, and this sequence begins about 2 weeks following the stage at which the ablations were performed.

In treated embryos, normal swimming behavior failed to develop. Under conditions which induced swimming behavior in control animals, treated animals produced weak dorsal-ventral flexions of the head and tail, but never produced the normal sinusoidal movement characteristic of swimming. However, within minutes after being injected with serotonin, these abnormal leeches swam normally. Thus, the neural circuitry underlying swimming had developed, but the expression of the behavior was disrupted in the absence of serotonin. The neural circuitry responsible for swimming therefore developed in the absence of 1) neurons containing a neurotransmitter essential for the expression of the behavior, and 2) the normal developmental sequence of swimming movements and resultant patterned sensory feedback.

Although the ablations were performed 2 weeks before swimming behavior developed, the serotonin-containing neurons were developing and contained serotonin for several days prior to the ablations. It is still possible that early events in swimming circuit development, preliminary to actual behavioral development, may be influenced by the absence of these neurons. Ablations of the serotonin-containing neurons around the time they first contain serotonin are currently being performed to address this possibility.

9.8 DENDRITIC GROWTH AND REORGANIZATION OF NEURONAL INTERACTIONS DURING INSECT METAMORPHOSIS, R.B. Levine and J.W. Truman. Dept. of Zoology NJ-15, Univ. of Wash., Seattle WA. 98195.

The nervous systems of holometabolous insects such as the moth, Manduca sexta, must mediate different behavior at different stages of life. They are therefore subject to a dramatic reorganization during metamorphosis. Our effort to understand this process has foccused upon the abdominal motorneurons, which we are able to identify as unique individuals. Metamorphosis is highly conservative with respect to these neurons, since all motorneurons present in the adult were also functional larval motorneurons. As described previously(Levine and Truman,Neuro. Abs. 6,1980), Some motorneurons die following the death of their targets after the pupal molt, but many survive the death of their larval target muscles and are retained to innervate new adult muscles. Larval neurons may possess bilateral or unilateral dendritic fields, depending upon the location of their target muscle. Regardless of larval morphology, however, all adult motorneurons possess bilateral dendritic fields even though this requires extensive growth on the part of unilateral neurons.

The behavior of the adult abdomen requires synergistic activity of motor neurons which were antagonists in the larva. This transformation is reflected in the synaptic inputs to bilaterally homologous motorneurons. In the larva for example, the motorneuron MN-1 has a unilateral dendritic field and is directly excited by an ipsilateral stretch receptor, while its contralateral homologue is indirectly inhibited. In the adult, both motorneurons have attained a bilateral dendritic tree and are directly excited by the same stretch receptor. The formation of the new excitatory pathway during adult development is correllated with dendritic growth of MN-1 in the region of the contralateral stretch receptor. Aquisition of common synaptic inputs by bilaterally homologous adult motorneurons is accomplished in this example not by growth of the presynaptic cells, but rather by elaboration if the motorneuron dendrites into areas occupied by its contralateral homologue.

The new excitatory pathway from stretch receptor to contralateral MN-1 forms during the latter part of adult development, and for a time coexists with the larval inhibitory pathway. Just prior to adult emergence stimulation of the receptor co-activates both pathways, but the inhibitory connection dominates. Within 30 minutes of emergence the inhibitory pathway has lost its influence on MN-1, and stimulation of the receptor causes pure excitation of the contralateral MN-1. The retension of the larval inhibitory pathway allows ongoing pupal behavior to persist undisturbed as the new excitatory pathway forms. Its rapid loss coincides with the onset of adult behavior patterns.
(Supported by grants from NIH and the McKnight Foundation).

9.9 HORMONAL CONTROL OF STAGE-SPECIFIC ECDYSIS MOTOR PATTERNS DURING LARVAL AND PUPAL DEVELOPMENT IN MANDUCA SEXTA. Janis C. Weeks and James W. Truman. Dept. Zoology, Univ. of WA, Seattle, WA 98195.

The tobacco hornworm M.sexta molts 6 times between hatching and adulthood. The first 4 molts occur between larval instars, the next molt is from larva to pupa, and the final molt is to the adult moth. Each molt culminates in the shedding of the old cuticle, an event called ecdysis(or eclosion, in the case of adult emergence). Each ecdysis is accomplished by rhythmic, anteriorly-directed peristaltic abdominal movements which may be accompanied by stage-specific limb movements during stages when limbs are present. These behaviors propel the old cuticle posteriorly along the body to be cast off. This process takes 5 - 15 min. and depending on the animal's stage may be preceded by a period of stereotyped rhythmic pre-ecdysis behavior which loosens the old cuticle. These behaviors are normally triggered by a 8500 mw peptide hormone, eclosion hormone (EH), which is released from the CNS into the blood roughly 0.5 - 1.5 hour before ecdysis begins, or they can be elicited prematurely by injecting insects with EH extract. To examine the neuronal basis of these hormone-mediated behaviors, we developed a semi-intact, deafferented preparation which responds to EH extract by producing the appropriate stage-specific pre-ecdysis and ecdysis motor patterns. Using standard intracellular and extracellular recording techniques on this preparation, and by filming and making EMG recordings from intact insects, we have characterized the larval and pupal motor patterns of M.sexta. In particular, we have determined key features of the patterns which are diagnostic of each stage;e.g., during larval ecdysis the abdominal prolegs show a strong protraction-retraction cycle during peristalsis, whereas at pupal ecdysis these appendages have been lost and this behavior is absent. Despite such changes, however, the same hormone (EH) turns on the motor patterns at each ecdysis. The characterization of these normal motor patterns has provided the framework for examining the hormonal control of circuitry modification during the larval to pupal transformation. By treating final instar larvae with a juvenile hormone analog (ZR515) at critical times during the ecdysone-mediated pupal transformation, we generated "larval-pupal intermediates" which have varying degrees of mixed larval and pupal character in their body structures, nervous systems, and behaviors. Notably, we produced animals which generated inappropriate motor patterns at their next ecdysis. Electrophysiological and neuroanatomical investigation of these animals should provide insight into how CNS circuitry is modified hormonally during development.

9.10 TRANSPLANTED SENSORY NEURONS IN THE CRICKET TEST THE SIGNIFICANCE OF SOMATOTOPY. Jonathan Bacon and R.K. Murphey, Department of Biology, SUNY, Albany, NY 12222.

Last year in this space we reported that cercal wind-sensitive filiform neurons form a somatotopic projection within the cercal glomerulus of the terminal abdominal ganglion of crickets. The filiform hairs are of two types: "T" hairs (vibrating transversly to the long axis of the cercus) are located dorsal and ventral on the cercus and "L" hairs (vibrating longitudinally) are found medial and lateral. A single afferent neuron innervates each hair and the location of its terminal arborisation within the glomerulus is largely a function of the hair's circumferential position on the cercus: "T" hairs project dorso-laterally in the glomerulus and "L" hairs project ventro-medially.

Two large interneurons, MGI and LGI, receive predominantly "T", and not "L" hair, excitatory input to their major dendrites. These dendrites are situated in positions in the glomerulus where direct contact with "T" hair afferents is more likely. We therefore find a correlation between synaptic connectivity of sensory afferents with these interneurons and their dendritic location within the afferent projection.

However this correlation does not prove that these morphological relationships are determinants of connectivity. A direct way to investigate this is to perturb the relationship of the somatotopic cercal projection to the interneurons. To do this, we have employed the experimental paradigm first described by Edwards and Sahota (J. exp. Zool., 166:387, 1967) in which a cercus is transplanted to the mesothoracic leg stump and the filiform hairs generate sensory projections to the mesothoracic ganglion. Our observations show that the ingrowing neurons produce a well defined glomerulus. "T" hairs tend to project laterally (as they do in the terminal ganglion) and "L" hairs project more ventrally and medially: thus a somatotopic map is recreated in foreign neuropile. Since MGI and LGI (whose axons are situated in the Ventral Intermediate Tract) have medially directed branches in the thoracic ganglia, this manipulation alters the relationship of the filiform afferents to the giant neurons. "L" hair afferents, rather than those from "T" hairs, are now more likely to form direct contact with MGI and LGI. However stimulation of the ectopic cercus produced no spiking activity in these interneurons though other ascending units were activated by the stimulation. Support for the hypothesis that somatotopy is an important determinant of connectivity would now be provided by the demonstration that "L" hair afferents provide sub-threshold inputs to the giant neurons in the thoracic ganglia. We are at present recording intracellularly from the giants in the thorax to test this possibility.

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9.11 NEURITE OUTGROWTH AND SYNAPSE FORMATION BY IDENTIFIED APLYSIA NEURONS IN DISSOCIATED CELL CULTURE. S. Schacher*, E. Proshansky*, and J. S. Camardo*. (SPON: R. Ambron.) Center for Neurobiology & Behavior, Depts. of Anatomy, Physiology, and Psychiatry, Columbia University, P & S, and N.Y. State Psychiatric Institute, New York, N. Y. 10032.

Dissociated cell culture techniques applied to identified central neurons of known properties and interconnections can provide an excellent system for examining factors which influence neurite outgrowth and specific synapse formation. We have succeeded in growing identified neurons from the abdominal ganglion of Aplysia californica in dissociated cell culture and have found that: 1) cells isolated with their axons remain essentially unipolar whereas cells without their original axonal process sprout numerous 'processes' each consisting of a fascicle of smaller neurites, 2) the number of fascicular sprouts and their thickness is dependent on the level of Aplysia hemolymph in the growth medium, and 3) the cholinergic interneuron L10 forms appropriate chemical synapses with three different follower cells.

Following enzymatic and mechanical dissociation, identified Aplysia neurons will attach and begin sprouting processes within 12 hrs when grown in medium containing Aplysia hemolymph. The nature of the neuritic sprouting is dependent on both the presence or absence of a cell's original axonal process and the level of Aplysia serum in the growth medium. When the initial axon is present, all neuritic sprouts emerge from the axon, leaving the cell in its original unipolar configuration. In contrast, cells lacking their original axon sprout numerous (5-15) processes from their cell bodies. At the electron microscope level, each 'process' consists of a fascicle of 4-10 smaller processes. The number of fascicular processes is reduced and their thickness increased when the level of Aplysia serum in the growth medium is reduced from 50% to 10%.

The cholinergic interneuron L10 will form chemical synapses with three different follower cells (LUQ, R15, and L7) by three days. The sign of the PSP is appropriate for each cell, so that firing of action potentials in L10 produces an IPSP in the LUQ cell, an EPSP in R15, and an EPSP-IPSP in L7. The chemical nature of the synapses was evident from the fact that there was no electrical connectivity, and that changing the membrane potential of the postsynaptic cell changed the magnitude of the PSP. The PSPs observed in culture tend to have prolonged rising and decay phases, and often repetitive firing of the presynaptic neuron is required to elicit a measurable response. Our data indicate that an identified neuron will form appropriate connections in culture with its usual target cells. It is not yet clear, however, to what extent this specificity is maintained, since it appears that cells may occasionally form synaptic connections which are not present in the ganglion.

9.12 THE ROLE OF ACTIVITY IN NEURONAL PLASTICITY IN THE COCKROACH S.F. Volman and J.M. Camhi. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In the escape system of the cockroach *P. americana*, the normal behavior is to turn away from a wind source. The sensory organs for wind are the cerci, a pair of abdominal appendages covered with fine sensory hairs. When one of the cerci is cut off, animals make directionally specific mistakes in their escape behavior. After about 4 weeks, without regeneration, the behavior is substantially corrected. There is also a concomitant increase in the ability of afferents from the intact cercus to evoke action potentials in directionally selective giant interneurons (GI's) on the contralateral (deafferented) side, which helps to restore the GI's normal responses (Vardi and Camhi, Neurosci. Absts., 6).

Is the ability of the contralateral cercal afferents to mediate changes in behavior and in the nervous system dependent on their activity during the 4-week "recovery" period? To answer this question, we compared the behavioral and physiological responses of three groups of unilaterally deafferented animals: 1) deafferented one day before testing, 2) deafferented for 4 weeks, and 3) deafferented with the intact cercus covered for at least 4 weeks. To prepare the last group, we cut off one cercus from last instar nymphs, and covered the other cercus with adhesive to immobilize the hairs. When animals molted 28-40 days later, they had a new set of sensory hairs and could be tested with wind stimuli. Covered animals did not show corrected behavior after molting (Volman et al., Neurosci. Absts., 6). The lack of behavioral recovery was not due to damage caused by the adhesive because animals having an intact cercus covered with plastic tubing also did not have corrected behavior after 4 weeks.

Intracellular recordings were made from the 3 largest ventral GI's in response to wind on the contralateral cercus. We plotted the receptive fields as the number of action potentials vs. wind-stimulus angle. For all 3 GI's, the responses in animals whose cerci had been covered were smaller than those in animals not covered during the 4 weeks after deafferentation; in particular, responses were smaller for frontal winds which had produced the most mistakes in behavioral tests. However, the responses were also significantly greater than those in animals which had been deafferented only one day before recording.

These results suggest that the long term level of activity can influence the amount of change in the ability of the contralateral afferents to drive the GI's. However, specific wind-evoked activity is not necessary to get some degree of change.

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9.13 HORMONAL CONTROL OF REGENERATION IN HYDRA

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We use hydra as a model system to understand how pattern formation, cellular growth and differentiation are controlled at the molecular level. We have found that four substances influence head and foot formation in hydra: an activator and an inhibitor of head formation and an activator and an inhibitor of foot formation. The two inhibitors are small molecules with molecular weights below 500 daltons, they have an overall positive charge, and they do not contain peptide bonds. The two activators have an overall negative charge, both are peptides, the foot activator has a molecular weight between 500 and 1000 daltons and the head activator of 1124 daltons. The head activator consists of 11 amino acids and has the sequence pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe. All four substances act at concentrations below 10^{-8} molar and their action is specific. At such low concentrations the head factors only influence head formation and the foot factors foot formation. Within the animal all four substances occur as gradients of sources, the head factors with a maximum in the head, the foot factors with a maximum in the foot region. The release from the structure-bound form is specific, and it determines the polarity of the tissue. Thus release of head factors is necessary to induce head regeneration, and that of foot factors to induce foot regeneration. In normal hydra all four substances are produced by and stored in nerve cells. We try to understand, how these substances act and interact to create the spatial and temporal pattern of growth and differentiation typical for hydra.

10.1 FURTHER CHARACTERIZATION OF BRAIN DOPAMINE RECEPTORS BY PHOTO-AFFINITY LABELLING AND DISC GEL ELECTROPHORESIS. K. Moroi* and L. L. Hsu (Spon: E. S. Barratt) Dept. of Psychiatry and Behavioral Sciences, Univ. of Texas Med. Branch, Galveston, Texas 77550.

We have previously reported on the purification and characterization of a dopamine (DA) receptor protein in brain synaptosomal membranes by affinity chromatography and disc gel electrophoresis. This purified DA receptor showed two polypeptide bands on SDS gel with apparent molecular weights of 40 K and 70 K daltons. In this study, we have examined the ligand specificity and binding properties of this DA receptor protein in rat synaptosomal membranes as well as in the microsomal fraction of the rat cortex using photo-affinity labelling and gel electrophoretic analyses. Crude mitochondrial membrane fraction including synaptosomes (P_{2M}) was prepared from rat cerebral cortex according to the procedure of De Robertes et al (J. Biol. Chem. 242:3489, 1967). Aliquots of P_{2M} were incubated with labelled DA, apomorphine (APO), spiroperidol, (SPD) haloperidol, (HAL), serotonim (5-HT) or acetylcholine (ACh) at 37°C for 40 min. in the absence or presence of fluorescent light. At the end of each incubation, an appropriate amount of cholic acid was added to the incubation mixture to a final concentration of 0.2% to solubilize the ligand labelled protein and this mixture was subsequently subjected to disc gel electrophoretic analysis. In another experiment, aliquots of cholate extract from P_{2M} were incubated with various labelled ligands described above and the incubation mixtures were subjected to disc gel electrophoretic analysis directly. After gel electrophoresis, one gel was stained with comassie blue R-250 for protein visualization and other gels were sliced into 12 equal pieces from origin to the dye marker. Each gel slice was mashed in a scintillation vial containing 1 ml of ethanol and 4.5 ml of Scintiverse counting solution for measurement of radioactivity.

Results indicated that, among all the ligands examined, only DA showed a radio binding peak at the protein band with a R_f value equivalent to the purified DA receptor protein. Furthermore, fluorescent light enhanced the DA binding peak in P_{2M} fraction 3 fold and the cholate extract showed much less photo affinity DA binding than P_{2M} pre-incubated with labelled DA under fluorescent light and subsequently extracted with cholic acid. The microsomal fraction showed similar results. Therefore this fast moving DA receptor protein seems to have ligand specificity towards DA only under our assay conditions. Whether it requires other components for SPD or HAL binding remains to be determined. The observation that cholate extract of P_{2M} exhibited much less photo-affinity labelling suggests that certain lipid component(s) in the P_{2M} membrane may be required for such irreversible DA binding. (This work was supported by a grant from R. A. Welch Foundation)

10.2 GTP-INDEPENDENT AND Na-DEPENDENT STEREOSPECIFIC BINDING OF ^{3}H-LISURIDE TO STRIATAL DOPAMINE RECEPTORS. P.F. Spano*, H. Usumaki*, S. Govoni, M. Memo*, V. Covelli, M. Carruba*, M. Trabucchi. (Spon: I. Hanbauer). Dept. of Pharmacology and Pharmacognosy, Univ. of Cagliari and Univ. of Milan, Italy.

Behavioral, pharmacological and clinical evidences indicate that lisuride can directly stimulate dopamine (DA) receptors in the brain and in the anterior pituitary. In vitro experiments using ^{3}H-lisuride indicate that this compound binds to central DA receptors. However, lisuride unlike apomorphine does not stimulate DA-sensitive adenylyl cyclase activity in rat striatal homogenates. To determine whether lisuride may interact with a specific subtype of DA receptors we investigated the effect of GTP and NaCl on ^{3}H-lisuride specific binding to rat striatum. The specific binding of ^{3}H-lisuride (26 Ci/mmol) to striatal membrane preparations was defined by 5×10^{-7} M d-butaclamol and appears to be saturable and stereospecific, both in presence or in absence of NaCl. In presence of 120 mM NaCl the B_{max} is increased by 40% (from 723±73 fmol/mg prot. to 1,035±80 fmol/mg prot.) and the K_D values remain unchanged. (1.8±0.4 nM without NaCl and 1.9±02 with NaCl). On the contrary, GTP up to 50 µM did not affect ^{3}H-lisuride binding characteristics in presence or in absence of NaCl. These findings are in contrast to those showing that the affinity of ^{3}H-apomorphine binding was decreased by one half in the presence of GTP.

The lack of effect of GTP on ^{3}H-lisuride binding characteristics seems to rule out the possibility that this ergot derivative is an agonist acting at the DA receptors linked to adenylyl cyclase (D_1). Moreover, the Na-dependency of ^{3}H-lisuride binding indicates that lisuride may bind to a Na-sensitive DA receptor (D_2).

10.3 RECOGNITION OF [^{3}H]SEROTONIN BINDING SITES IN RAT BRAIN BY ANALOGUES OF TRYPTAMINE. D. L. Nelson, W. Taylor* and B. Weck*. Dept. of Pharmacology & Toxicology, Col. of Pharmacy, Univ. of Arizona, Tucson, AZ 85721

In this study a series of structural modifications at the aminoethyl portion of tryptamine (TRYP) were examined to determine their effects on recognition by [^{3}H] serotonin ([^{3}H]5-hydroxytryptamine, [^{3}H]5-HT) binding sites in rat brain, i.e., those sites commonly classified as 5-HT_1 receptors. A standard ligand-binding assay was used to measure the binding of [^{3}H]5-HT to membranes prepared from rat cortex (Pedigo et al., J. Neurochem. 36: 220, 1981) except that ascorbate was not used and the antagonist metergoline (1 µM) was used to define nonspecific binding. Removal of the 5-hydroxy group of 5-HT to form TRYP resulted in a large decrease in potency at the 5-HT_1 sites (IC_{50}5-HT = 3-5 nM, IC_{50}TRYP = 100-200 nM). In addition, 20-25% of these [^{3}H]5-HT binding sites were resistant to inhibition by TRYP. This suggested that the 5-hydroxy group was necessary for recognition by these sites. However, alkylation of TRYP to form dimethyltryptamine (DMT) or diethyltryptamine (DET) resulted in compounds which could now inhibit all specific [^{3}H]5-HT binding. The inhibition curves produced by DMT and DET were shallower than that produced by TRYP, and while the IC_{50} values for the three compounds were very similar, at low concentrations (3-10 nM) DMT and DET appeared to be more potent inhibitors of [^{3}H]5-HT binding than TRYP. Synthesis of an analogue having even greater bulk on the amino group, i.e., diisopropyltryptamine (DIPT), produced a compound which had an overall potency that was much less than TRYP (IC_{50}DIPT = 800-1500 nM). However, this compound produced a biphasic curve for the inhibition of [^{3}H]5-HT binding such that at low concentrations (3-10 nM) it was more potent than TRYP while at higher concentrations (100-3000 nM) it appeared less potent than TRYP. Syntheses of compounds with more complex substitutions at the amino group were also carried out. When the amino group was replaced by a piperidyl or a morpholinyl group the resulting compounds produced inhibition curves which were essentially identical to DIPT. The present data indicated that modifications in the structure of 5-HT could significantly alter recognition by 5-HT_1 sites, resulting in discrimination between different populations of these sites. TRYP, DMT and DET are commonly classified as 5-HT agonists, and there is now preliminary evidence that DIPT also acts as an agonist. If high affinity [^{3}H]5-HT binding truly represents a class of receptors, then these data suggest the possibility of synthesizing 5-HT agonists which are relatively specific for certain 5-HT receptor subtypes. (Supported by NIH grant NS16605 and a PMA grant.)

10.4 COMPUTER-ASSISTED MICRODENSITOMETRY OF SEROTONIN RECEPTOR SUBTYPES IN RAT CENTRAL NERVOUS SYSTEM. P.P. Deshmukh, D.L. Nelson, A.S. Marathay* & H.I. Yamamura. Dept. of Pharmacology & Optical Sciences Center, University of Arizona, Tucson, AZ 85724

Computer-assisted image processing to analyze autoradiograms can provide precise quantitation of neurotransmitter receptors in micron-sized regions of the brain. In this study we have used computerized microdensitometry to analyze subtypes of serotonin receptors in rat central nervous system. A number of recent studies have proposed at least two broad classes of serotonin (5-hydroxytryptamine, 5-HT) receptors. One class, the 5-HT_1 receptor, is defined by the high affinity binding of [^{3}H]5-HT and the other, the 5-HT_2 receptor, is defined by the high affinity binding of [^{3}H]spiperone. It has also been shown that the 5-HT_1 sites can be further subdivided into two groups according to their affinity for the neuroleptic spiperone. These subtypes are called 5-HT_{1-A} (having high affinity for spiperone) and 5-HT_{1-B} (having low affinity for spiperone). The binding of ^{3}H-5-HT to rat brain slices was performed essentially as described by Young and Kuhar (Eur. J. Pharmacol. 62:237, 1980). Serial sections were incubated to give: 1) total 5-HT binding (in the presence of 2nM ^{3}H-5-HT); 2) non-specific binding where ^{3}H-5-HT is displaced by the addition of 1 µM metergoline and 3) binding to 5-HT_{1-B} sites where binding to 5-HT_{1-A} sites has been blocked by the presence of 1 µM spiperone in the incubating medium. After proper exposure and development the autoradiograms obtained on LKB-ultrofilm were scanned with a Perkin-Elmer microdensitometer that scans 65000 lines with 1024 pixels per line. A scanning aperture of 5 µm was used in this procedure. A Hewlett-Packard 2020 Tape drive with a 7-track reel tape was used to record the scanned information. This 7-track tape was then converted to a 9-track, 800 bits per inch format at the University of Arizona computer center. This 9-track tape was then processed at the Digital Image Analysis Laboratory (DIAL) where there is built-in software available for picture processing and display of processed images. A hard copy in psuedocolor can be produced on polaroid print film or on a 35mm Ektachrome format. Image processing brings out very subtle changes in receptor densities in minute areas that are too small for measurement by binding to homogenates. For example, septum and inner layers of frontal cortex show a preponderance of 5-HT_{1-A} sites while in the CA_1 area of the hippocampus and superficial layers of the superior colliculus the 5-HT_{1-B} sites predominate. The use of this technique should therefore, prove very useful for mapping and characterizing 5-HT receptor subtypes as well as other receptors within the central nervous system. (Supported by NIMH grant MH36861, H.I.Y. is supported by a RSDA from the NIMH.)

10.5 QUANTITATIVE AUTORADIOGRAPHY OF [^{3}H] DESMETHYLIMIPRAMINE AND [^{3}H] IMIPRAMINE BINDING SITES. T.C. Rainbow and A. Biegon. The Rockefeller University, New York, NY 10021.

We have used the recent LKB Ultrofilm method of autoradiography to localize high affinity binding sites for [^{3}H] desmethylimipramine (DMI) and [^{3}H] imipramine (IMI) in rat brain. The antidepressants DMI and IMI are, respectively, potent inhibitors of norepinephrine (NE) and serotonin (5HT) re-uptake, which recently have been shown to bind to high affinity sites in brain tissue. Our procedure was to label frozen 32μ thick brain sections in vitro with [^{3}H] DMI, [^{3}H] IMI or [^{3}H] nitroimipramine (NI), an irreversible inhibitor of 5HT uptake (Rehavi et al., Biochim. Biophys. Res. Comm. 99 1981). Brain sections were incubated at 4° with 1-10nM of [^{3}H] ligands in 50mM Tris buffer containing 300mM NaCl. Non-specific binding was measured by co-incubation with 100μM unlabeled DMI. The labeled sections were placed against LKB Ultrofilm as described (Rainbow et al., J. Neurosci. Methods 5 1982) to generate autoradiograms. The optical density readings were converted into the amount of binding site per mg of protein with tritium standards. We also analysed autoradiograms with computerized image processing. The properties of [^{3}H] DMI and [^{3}H] IMI binding to frozen sections corresponded closely to previous reports of high affinity tricyclic binding to membrane preparations. In sections removed for scintillation counting, each ligand was observed to bind to a single, specific site, with half-maximal binding occurring at 4-6nM.

The distribution of [^{3}H] tricyclic sites was in striking accord with the known distribution of monoamine terminals in rat brain. The anatomical location of DMI binding sites closely paralled the location of NE terminals, with high concentrations of binding sites found in the locus coeruleus, the ventral portion of the nucleus stria terminalis, the anterior ventral thalamus and the dorsomedial nucleus of the hypothalamus. There were low concentrations of DMI binding sites in the caudate putamen and substantia nigra, regions with a low content of NE. By contrast, the distribution of IMI and NI binding sites was in good agreement with the location of 5HT terminals in rat brain, with a high density of binding sites observed in the olfactory tubercle, the interpeduncular nucleus, the raphe nuclei and the nucleus of the facial nerve. There was a low content of IMI or NI binding sites in the ventral thalamus, a region with a low level of 5HT.

Our results support the hypothesis that the DMI and IMI binding sites correspond to some aspect of the presynaptic uptake site for monoamines and suggest that monoamine neurons might be important target sites for the clinical actions of antidepressants. It may be possible to use in vitro autoradiography with [^{3}H] DMI or IMI to quantify monoamine terminals in brain sections.

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10.6 BINDING TO A SUBPOPULATION OF ^{3}H-IMIPRAMINE SITES BY ^{3}H-Ro 11-2465, A POSSIBLE IRREVERSIBLE LIGAND. A. Dumbrille-Ross and S.W. Tang. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T 1R8.

In order to examine whether the compound Ro 11-2465 (Ro) (a CN-derivative of imipramine) and imipramine (IMI) bind to the same site, characteristics of receptor binding of ^{3}H-Ro were examined in both rat cerebral cortex homogenates and human platelets. Competition of ^{3}H-Ro binding by various drugs, effects of raphe lesioning, sodium and temperature dependence of ^{3}H-Ro binding was examined and compared to that of ^{3}H-IMI binding.

Serotonin was the only neurotransmitter which competed for binding of both ^{3}H-Ro and ^{3}H-IMI (Ki 1.3 μM and 0.4 μM respectively). Drugs competed for the binding of both ligands with an order of potency similar to that in blocking serotonin uptake. Binding of ^{3}H-Ro and ^{3}H-IMI, similar to serotonin uptake, is sodium dependent. Raphe lesions decrease binding of both ^{3}H-Ro and ^{3}H-IMI.

^{3}H-Ro binds (23°) to cortical tissue with a K_d of 0.25 nM and B_{max} 250 fmol/mg protein. ^{3}H-IMI (4°) binds with K_d of 7.5 nM and B_{max} of 328 fmol/mg protein. In human platelets, ^{3}H-Ro binds to about 50% of the number of sites bound by ^{3}H-IMI (B_{max} 311 fmol/mg protein and 605 fmol/mg protein respectively). Binding characteristics of ^{3}H-Ro and ^{3}H-IMI are different at 4° from 23°. The B_{max} of ^{3}H-IMI decreases about 50% when incubated from 20 min to 24 hr at 23°, suggesting that a discrete population of sites may be susceptible to temperature degradation. However the B_{max} of ^{3}H-Ro binding is not temperature dependent, and the association time at 4° is slow, about 8 hr. If the tissue is incubated with ^{3}H-Ro at 4° for 16 hr, the binding becomes largely irreversible. After binding ^{3}H-Ro to cortical homogenates (4°) and thoroughly washing off the unbound ^{3}H-Ro, binding of ^{3}H-IMI is reduced by about 50%.

These results suggest that ^{3}H-Ro and ^{3}H-IMI label sites of similar characteristics with high affinity, and that ^{3}H-Ro may label a subpopulation of ^{3}H-IMI binding sites.

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10.7 PICROTOXIN BINDING SITES INTERACT WITH BOTH CATION AND ANION SITES IN BENZODIAZEPINE/GABA RECEPTOR COMPLEXES, R.F. Squires and E. Saederup*. Rockland Research Institute, Orangeburg, NY 10962.

Several picrotoxin-like convulsants increase the number of brain specific ^{3}H-flunitrazepam (^{3}H-FLU) or ^{3}H-CGS 8216 binding sites protected against heat inactivation (30 min, 60°C) in 200 mM NaCl. These convulsants include picrotoxin (EC_{50}=16 uM), anisatin (3.6), isopropylbicyclophosphate (15), isopropylbicyclophosphothionate (1.8), t-butylbicyclophosphate (4.4), tetramethylene disulfotetramine (51) and picrotoxinin (25). Picrotin was inactive at 100 uM. Picrotoxin decreased the concentration of NaCl required to provide 50% of its maximum protective effect and increased the Hill number for NaCl from 2 to 3. Hill numbers greater than one indicate positive cooperativity between separate binding sites, possibly for Na^+ and Cl^-, respectively. Picrotoxin (100 uM) and 5 mM $CaCl_2$ combined, but not individually, provide significant protection against heat inactivation, which is synergistically enhanced by 50 mM NaCl. Dialysis of rat brain P2 membranes, first against EDTA, then water, removes about 90% of the GABA which cannot be removed by dialysis against water alone. ^{3}H-FLU and ^{3}H-CGS 8216 binding site in such EDTA-water dialyzed membranes are partly protected against heat inactivation by GABA plus $CaCl_2$, but by neither substance individually. In the presence of 500 uM GABA, $CaCl_2$ provides 50% of its maximum protective effect near 600 uM. Mg^{2+}, Ba^{2+}, Sr^{2+} and Mn^{2+} also provide similar protection in the presence of GABA while eight other divalent cations were inactive. In the presence of 5 mM $CaCl_2$, ten GABA-A receptor agonists (β-aminopropane sulfonate, β-guanidinopropionate, imidazole acetate, muscimol, isoguvacine, piperidine-4-sulfonate, THIP, trans-4-amino crotonate, dihydromuscimol and d,l-beta-homoproline) can partly or entirely replace GABA with EC_{50} values in the 5-150 uM range. Using ^{3}H-FLU as radioligand, time courses of heat inactivation at 60°C in the presence of saturating $CaCl_2$ and GABA-A receptor agonists are polyphasic with slow components constituting about 45% of unheated control binding (Bo) for THIP, isoguvacine and P4S, 56% for imidazoleacetate, β-guanidinopropionate and aminopropane sulfonate, and 68% for GABA and muscimol, suggesting the presence of at least 4 subpopulations of BZ receptor complexes. Using ^{3}H-CGS 8216 the corresponding Bo value in the presence of 5 mM $CaCl_2$ and 500 uM GABA is 95%, suggesting that this ligand preferentially labels a subpopulation of ^{3}H-FLU binding sites. Picrotoxin does not modify the ability of GABA-A receptor agonists to enhance the protective effects of NaCl or $CaCl_2$. Taken together, these results indicate the presence of independent binding sites for cations, anions, GABA, picrotoxin and benzodiazepines in binding-site complexes.

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10.8 LOCALIZATION OF RECEPTORS ON CHEMICALLY IDENTIFIED NEURONS IN CULTURE. Louis Terracio*, A.J. Beitz, W.E. Wells, and James Buggy (SPON: John J. Freeman). Depts. of Anatomy and Physiology, Univ. of S. Carolina, Colurbia, S.C., 29208.

The localization of specific receptors on chemically defined neurons within specific brain regions would be useful in addressing many problems in neurobiology. We have devised a technique using primary cultures of rat brain that permits characterization of neurons by immunohistochemistry and radiohistochemistry. Neuronal cells were isolated from 1-3 day old rat cerebellum (CER), brain stem (BS), and cerebral hemispheres (CH), using both mechanical and enzymatic digestion procedures. The isolated cells were plated at high density on glass coverslips with F12K plus 10% horse and calf sera and fed on days 4-7 in vitro with F12K-10HS-10CS plus 10mg/ml cytosine arabinoside. The resulting cultures from each of the three brain regions were composed of several populations of neuronal-like cells with few contaminating glial or fibroblast cells. Cultures from each region were first characterized immunohistochemically. Cultures of CH were tested for cholecystokinin (CCK)- and somatostatin (SOM)-like immunoreactivity. BS neurons were reacted with met-enkephalin (ME), serotonin (5HT) and neurotensin (NT) antisera, while CER cultures were reacted with angiotensin (AII) antisera. CH contained distinct populations of neurons that were positive for SOM and CCK. BS possessed neurons that were positive for 5HT, NT, and ME, while CER contained a population of neurons that had AII-like immunoreactivity. Receptor-binding autoradiography was performed using modifications of the procedure of Young and Kuhar (1979). Opiate receptors were demonstrated using ^{3}H-naloxone, β-adrenergic receptors (β-Ad) using ^{3}H-dihydroalprenolol (DHA) and muscarinic-cholinergic receptors (MCR) using ^{3}H-quinuclidinyl benzilate (QNB). β-Ad and MCR were demonstrated on neurons from all three regions. Opiate receptors were found on some neurons from BS and CH. The combined localization of peptides or neurotransmitters and receptors was accomplished for the ligands QNB and naloxone by first performing the radiohistochemical procedure followed by fixation of the cultures prior to immunohistochemistry. The fixation step did not retain DHA binding and therefore DHA radiohistochemistry was performed after immunohistochemistry. The results of this combined procedure indicate that in the CH cultures neurons could be found that had both SOM and β-Ad. In BS cultures there were neurons that possessed NT and opiates, NT and MCR and a few neurons with 5HT and opiates but no neurons with 5HT and MCR. The results of this study indicate that this combined technique may be a useful approach to characterizing neurons both chemically and pharmacologically from a variety of discrete brain regions.

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10.9 BENZODIAZEPINE RECEPTORS ARE NOT EXCLUSIVELY LOCATED ON PURKINJE CELLS: A STUDY WITH THE PCD MUTANT MOUSE. F. M. Vaccarino*, B. Ghetti, M. A. Rea, and M. H. Aprison. Depts. of Psychiatry, Pathology and Biochemistry, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

The benzodiazepine (BZ) action on the CNS has been linked to GABAergic mechanisms on the basis of biochemical and physiological evidence, however the distribution of the GABA and BZ receptors is not always parallel. In the cerebellar cortex of the mouse and rat, autoradiographic studies have shown that the BZ receptors are localized in the molecular layer, while GABA receptors are in the granular layer. To determine whether the binding noted in the molecular layer is associated only to Purkinje neurons and to measure the binding in a more quantitative manner, we used Purkinje cell degeneration (pcd) mutant mice. In these mice, Purkinje cells start to degenerate at 17 days of age until they are almost completely lost at 45 days. Granule cell loss begins after 50 days of age; morphological data in 1 year old pcd's indicate that more than 50% of the granule cells have degenerated. BZ binding in affected pcd mice, compared to control littermates, was measured at different ages with [^{3}H]Flunitrazepam (FNZ) and [^{3}H]ethyl β-carboline (BCC). Both ligands were employed at concentrations near to Kd. Since GABA and GABA agonists can increase the affinity at BZ receptors, we added Muscimol (10 μM) to maximally stimulate the latter receptors to avoid variations due to different levels of endogenous GABA. RO5-4864, an inactive BZ that binds to non-neuronal cells, is ineffective at 1 μM in displacing the above ligands, indicating that they are selective for neuronal cells. The binding data from affected pcd animals, 45 to 80 days old, show a 25% decrease for the two ligands; in animals 180 days old, the binding decreased by 45% for both ligands whereas in animals 300 days of age, FNZ and BCC binding decreased 50 and 60%, respectively. No difference in binding was detected at any age in the cortex and hippocampus of affected animals compared to controls. Scatchard analysis of BCC binding revealed that the above decreases were due to a loss of total receptor number without any change in their affinity. Scatchard plots of FNZ binding data appeared curvilinear, with a lower Bmax value in the affected animals. These results do not support an exclusive localization of BZ receptors on the Purkinje cell dendritic tree and suggest that this binding might also be associated with other cerebellar neurons (granule cells and/or neurons of the deep nuclei). -Supported in part by an International Rotary Foundation grant (F.M.V.) and a NIH research grant RO1-NS-14426 (B.G.).

10.10 SYNAPTIC LOCALIZATION OF SPECIFIC BINDING OF 125I-α-BUNGAROTOXIN IN THE RAT SUPERIOR CERVICAL SYMPATHETIC GANGLION. A. J. Smolen and T. Lindley*. Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

In the rat superior cervical sympathetic ganglion (SCG), α bungarotoxin (αBT) demonstrates binding that is saturable and inhibited by nicotinic ligands. However, αBT does not inhibit the physiological response of ganglionic neurons to preganglionic stimulation or to exogenously applied acetylcholine. Thus, the specificity of αBT for ganglionic nicotinic cholinergic receptors has been questioned. It has been proposed that in some regions of the central nervous system αBT may bind to cholinergic receptors without blocking transmission. To determine whether the morphological localization of the binding sites of αBT in the SCG is related to the distribution of cholinergic receptors sites, we undertook the present study.

Intact, desheathed SCGs were incubated for 30 min in 80nM 125I-αBT with or without 1mM of the nicotinic antagonist d-tubocurarine (d-TC). After the incubation, the ganglia were washed, fixed and assayed for bound radioactivity by gamma counting. These ganglia were subsequently processed for electron microscopic radioautography.

In the absence of d-TC, there was a total binding of 45 fmoles αBT/ganglion. With d-TC, the nonspecific binding was 18 fmoles/ganglion. The specific binding was calculated to be 28 fmoles/ganglion. For the electron microscopic analysis, a modification of the cross-fire method of Blackett and Parry was employed, in which the localization of real grains resulting from the non-specific and the specific binding was compared to a hypothetical grain distribution based on the geometrical properties of the tissue section. A computer minimizing routine was employed to adjust the relative weights of each of the potential sources of hypothetical grains until a "best-fit" with the real grain distributions occurred. The nonspecific binding of αBT was uniform across all tissue components, with the exception of a significant concentration on the membrane of the ganglion cell body. By contrast, the specific binding of αBT was highly localized to synaptic membranes and to a lesser extent to dendritic membranes.

The localization of the specific binding of αBT in the rat SCG is consistent with that of cholinergic receptors or with some other molecule located in the region of the synapse, and thus may be useful as a marker for the postsynaptic membrane.

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10.11 AUTORADIOGRAPHIC LOCALIZATION OF HIGH AND LOW AFFINITY MUSCARINIC CHOLINERGIC BINDING SITES IN THE BRAIN AND SPINAL CORD. J. K. Wamsley, M. A. Zarbin* and M. J. Kuhar. Dept. of Psychiatry, Univ. of Utah, Salt Lake City, UT 84132 and Dept. of Neuroscience, Johns Hopkins Univ., Baltimore, MD 21205.

High and low affinity muscarinic cholinergic binding sites can be discriminated on the basis of their differing affinities for muscarinic agonists (Birdsall et al., Mol. Pharmacol., 14: 723, 1978). Autoradiographic techniques for the microscopic localization of receptors have been applied to differentially localize high and low affinity muscarinic cholinergic binding sites (Wamsley et al., Brain Res., 200: 1, 1980). We have now utilized this technique to map the distribution of high vs. low affinity muscarinic cholinergic binding sites throughout the brain and spinal cord.

Low affinity muscarinic cholinergic binding sites predominate in the forebrain. Such structures as the cortex, caudate-putamen, hippocampus, amygdaloid nuclei, and many of the structures in the thalamus and hypothalamus contain mostly low affinity sites. High affinity muscarinic sites could be identified in the medial septum, preoptic region, anterior hypothalamus, thalamic reticular nucleus, and in the lateral habenula. Clearly, the high affinity muscarinic cholinergic binding sites predominate in brainstem regions. Most of the muscarinic receptors identified in the superior and inferior colliculi, raphe nuclei, structures on the floor of the fourth ventricle, medial vestibular nucleus, cochlear nuclei, and in the facial and hypoglossal cranial nerve nuclei, were of the high affinity type. Low affinity muscarinic agonist binding sites predominate in the pontine nuclei and in the substantia gelatinosa of the spinal trigeminal nucleus. The most dramatic separation of high and low affinity sites was found in the spinal cord where only high affinity sites exist in the ventral horn and only low affinity sites are found in the dorsal horn.

Observations in the peripheral nervous system indicate the high affinity site is located in ganglionic cell bodies and is being orthogradely transported, whereas the low affinity site is localized in neuron terminals and is undergoing retrograde transport (Wamsley et al., Brain Res., 217: 155, 1981); Zarbin et al., J. Neurosci., in press). We hypothesize the same relationship may exist in the brain. The localization of high affinity sites in motor cranial nerve nuclei and in several white matter pathways in the brain, would support this hypothesis. High affinity muscarinic cholinergic sites may thus represent a marker for cholinergic cell bodies. These receptors diminish in the ventral horn of the spinal cord in patients with amyotrophic lateral sclerosis (Whitehouse et al., in preparation).

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10.12 RECEPTOR-MEDIATED UPTAKE OF ACTH(1-24) IN RAT BRAIN SLICES. Frank S. LaBella, Gary Queen*, Douglas Stein* and Margaret Millar*. Dept. of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, Canada.

(Phe^2, norleu^4)- ACTH(1-24) was labelled with 125I, and monoiodinated tyr^{23}- ACTH isolated by HPLC. Buckley et al (Endocrinol. 109:5, 1981) report that monoiodinated (phe^2, norleu^4)-ACTH(1-38) is as fully active as the natural peptide. Brain slices (0.2 x 0.2 x 0.6 mm) were incubated with 0.4 nM labelled ACTH in Krebs-Ringer-bicarbonate at 37°, in the presence or absence of competing ligands. The slices were filtered on a nylon screen, washed with cold buffer, scraped from the screen and dissolved in KOH. Aliquots were analyzed for 125I and protein. Specific binding was resolved into two components, one with Kd 50 nM and another, Kd 5 uM. Specific binding of ACTH to slices reached a maximum at 1 hr, was enhanced by added glucose, and reduced by 80% in the presence of ouabain, NaF, or dinitrophenol. Dissociation of ACTH was 50% complete at 30 min in metabolically inhibited slices and only 30% at 2 hr in non-inhibited tissue, suggesting metabolically dependent sequestration (internalization?) of specifically bound ACTH. The number of high affinity binding sites for ACTH in whole brain, estimated in the presence of inhibitors of sequestration, was 0.4 pmol/mg protein, and 16 pmol/mg protein for the low affinity sites. Specific binding of ACTH in decreasing rank order: hypothalamus, striatum, hippocampus, cerebral cortex, cerebellum, midbrain, brainstem. Potency of ACTH fragments in displacing ACTH(1-24) in decreasing rank order: 1-24, 11-24, 1-10, 4-10. This order of fragment potencies is similar to that found by us for binding of ACTH(1-24) to dispersed adrenal cells. Of a large number of opiate drugs and hormonal peptides, only metenkephalin and, to a lesser extent, beta-endorphin competed with ACTH(1-24). Snell and Snell (FEBS Lett. 137:209, 1982) have pointed to structural similarities between the N-terminal region of ACTH and the receptor conformation of enkephalin. Rat liver slices showed about one-third the level of specific binding estimated in brain, whereas none was detected in slices of heart, kidney, spleen, lung and intestine. The brain slice preparation has the potential to yield information on the turnover of ACTH receptors, receptor density on the neuron membrane, and receptor coupling to the biological transducer. This slice system promises, also, to provide information on the molecular basis for a putative role of ACTH in tolerance and dependence to opiates which may not be available from studies with brain homogenates or membranes. (Supported by MRC of Canada).

11.1 LESIONS OF CEREBELLAR NUCLEI ABOLISH THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE RESPONSE. C.H. Yeo*, M.J. Hardiman*, M. Glickstein and I. Steele Russell*. MRC Unit on Neural Mechanisms of Behaviour, 3 Malet Place, London WC1E 7JG, U.K.

Extensive lesions of the cerebral cortex do not impair acquisition or retention of the classically conditioned nictitating membrane response (NMR) of rabbits (Oakley and Russell, Phys. Behav. 18: 931-937, 1977). A possible subcortical pathway is suggested by a recent study (McCormick et al. Bull. Psych. Soc. 18, 103-105, 1981) in which lesions of cerebellar cortex abolished the ipsilateral conditioned NMR.

The output of the cerebellum is exclusively via the deep cerebellar nuclei, so lesions of these nuclei alone should abolish the conditional response. We trained rabbits on daily sessions of 200 trials using 500 msec conditional stimuli of light or white noise interspersed in blocks through the session to give 100 daily trails of each. The unconditional stimulus was a brief, paraorbital electrical shock.

18 rabbits were pretrained with five sessions giving the shock to the right side. They were then subjected to suction or radio-frequency lesions of the right cerebellar nuclei, or control areas. Subjects were then given five training sessions to the right (ipsilateral) side followed by five sessions to the left (contralateral) side.

In six cases the lesion abolished conditional responses on the side ipsilateral to the lesion. All animals were unaffected contralaterally and unconditional responses were normal on both sides. Animals with abolished conditional responses had extensive damage to the interposed nuclei accompanied by some damage to medial parts of the dentate nucleus and small areas of overlying paramedian lobe. Damage to paramedian lobe and/or dentate nucleus alone failed to abolish conditioning.

We suggest that the interposed nucleus is a critical structure for NMR conditioning. If the cerebellar cortex is involved, then the relevant portion is most likely to be contained within the division C of Voogd.

11.2 EFFECTS OF ELECTROLYTIC LESIONS OF CEREBELLAR NUCLEI ON CONDITIONED BEHAVIORAL AND HIPPOCAMPAL NEURONAL RESPONSES. G. A. Clark*, D. A. McCormick*, D. G. Lavond*, K. Baxter*, W. J. Gray* and R. F. Thompson (SPON: K. A. Sigvardt). Dept. of Psychol., Stanford Univ., Stanford, CA 94305.

A critical issue for the biology of learning concerns the identification of brain structures essential for learning to occur. We report here that unilateral lesions of dentate and interpositus cerebellar nuclei abolish conditioned but not reflexive nictitating membrane (NM) responses in trained rabbits.

Rabbits were trained with standard procedures for classical conditioning of the NM response: a 350 msec tone conditioned stimulus (CS) was paired with a 100 msec airpuff to cornea, which elicited NM extension. Interstimulus interval was 250 msec, with a variable intertrial interval of 30 sec. Animals were trained to a behavioral criterion of eight conditioned responses out of any nine consecutive trials, then given one day of further training. Electrolytic lesions (2mA, 5 min.) were then made through mono- or bipolar electrodes previously implanted in dentate and interpositus cerebellar nuclei ipsilateral to the trained (left) eye. Animals were subsequently given four days of retraining, as well as a fifth session in which training was switched to the contralateral (right) side for the first half of the day, then switched back to the original (left) side.

In all animals (n=12) with damage to deep nuclei and surrounding fibers, lesions caused complete or near-complete abolition of conditioned responses on the ipsilateral side ($p < .001$). In contrast, lesions did not effect the amplitude of unconditioned responses to airpuff, indicating lesion effects were not due simply to motor disturbances or performance variables. When training was switched to the contralateral (right) eye, animals learned within the first few trials ($\bar{x} = 6$), but did not relearn when training was returned to the left (lesioned) side (cerebellar connections with other structures are primarily unilateral). The fact that animals learned the contralateral response so quickly rules out nonspecific lesion effects. Control animals in which lesions spared the deep nuclei showed no memory deficits.

Recordings of hippocampal neuronal unit activity during training indicated that nuclear lesions also abolished conditioned increases in neural activity normally evoked by the CS in this paradigm. As with the behavior, training on the contralateral side reinstated the conditioned neuronal response within the first few trials of training.

Present and previous studies thus indicate that disruptions of cerebellar circuitry at several points--cerebellar cortex, deep nuclei, and superior cerebellar peduncle--all abolish conditioned NM responses. Such convergence argues strongly that it is the cerebellum itself which is essential for the learned response.

11.3 BEHAVIORAL EFFECTS OF HIPPOCAMPECTOMY DEPEND ON INTER-EVENT INTERVALS. J.N.P. Rawlins*, J. Feldon* and J.A. Gray* (SPON: C. Blakemore). Dept. of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, England.

Large hippocampal lesions abolish the partial reinforcement extinction effect (PREE) in the straight alley, when 48 acquisition trials are given at 6 trials/day with an intertrial interval (ITI) of 4-8 minutes (Rawlins, J.N.P., Feldon, J. and Gray, J.A., Exp. Brain Res., 38:273, 1980). The development of a PREE depends in part upon the animal's ability to remember on a rewarded trial that he was recently non-rewarded (e.g. Capaldi, E.J. and Minkoff, R., J.Exp.Psychol., 81: 56, 1969). The present experiments reduced the time over which the rats were required to associate events. In one experiment on the PREE, the ITI was reduced to a few seconds. A second experiment directly paired non-reward and reward by presenting a reward only after a delay in the goal box on 50% of acquisition trials, and measured the increased resistance to extinction that this produces (the partial delay extinction effect).

Rats with large hippocampal lesions (HC), or cortical control lesions (CC), or rats having had sham operations (SO) were trained to run down a 170cm straight alley for food. 48 acquisition trials were used at 6 trials per day. In experiment 1, the rats were rewarded on a random 50% of trials. On rewarded trials the rats were confined in the goal box while they consumed the food, and were then replaced in the startbox for the next trial. On non-rewarded trials the rats were confined in the goal box for an equivalent time, and then replaced in the startbox. The ITI was thus less than 3 seconds. In experiment 2 the rats were confined as before on rewarded trials, and then replaced in a waiting box. On delay trials, the rats were confined without food as for the non-reward trials in experiment 1, but food was then dropped via a tube into the goal cup. The ITI was 4-8 minutes, spent in a waiting box.

Both the partial reinforcement and the partial delay schedules used produced an increase in resistance to extinction, which was not blocked by hippocampectomy. This is consistent with the view that hippocampal damage does not prevent the formation and use of associations, so long as the interval between the events which must be associated is short enough. However hippocampectomy may retard the formation of associations between events which are separated by longer inter-event intervals.

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11.4 SELECTIVE KAINIC ACID LESIONS OF THE HIPPOCAMPAL FORMATION: EFFECTS OF SUBICULUM VS. CA3 CELL LOSS ON PLACE AND CUE LEARNING. Leonard E. Jarrard. Dept. of Psychol., Washington & Lee Univ., Lexington, VA 24450.

Recent research demonstrating different behavioral effects resulting from selective damage to either hippocampal cell fields or projections prompted the present investigation of the effects of damage to CA3 cell field or subiculum on performance of a complex place and cue task. Using a within-subjects design, rats were trained before the operations to run on an 8-arm radial maze with a procedure that involved two kinds of learning (place and cue) and two memory functions [reference memory (RM) and working memory (WM)]. In the place version of the task the same 4 arms were baited from trial to trial and room cues (door, rack of cages, overhead lights) remained in the same spatial location. In the cue task 8 removable inserts of different materials were placed in the arms but the location was changed from trial to trial in a random order -- 4 of the 8 cues were consistently baited.

After learning the tasks the rats were divided into two control groups (operated and unoperated) and 4 lesion groups. Kainic acid (KA) was injected bilaterally into either the CA3 cell field, the subiculum, or the lateral ventricles. Rats in the remaining group received extensive damage to the hippocampus (including all cell fields, dentate gyrus, and fimbria) using an aspiration technique. Following recovery the animals were retrained to approach the last 4 arms (and 4 cues) that had been learned before the operations, and then reversal training was carried out.

Analysis of the behavioral data indicated an impairment on the place task but not the cue task in animals with subiculum cell loss and those with extensive damage to hippocampus. Even though rats in the CA3 Group suffered extensive loss of pyramidal cells in the CA3 cell field, their performance did not differ from controls on either the place or cue task. Rats receiving intraventricular injections of KA were impaired on both tasks but were able to relearn to successfully perform the cue task. Analysis of the error data broken down by error type indicated no differences between RM and WM for any of the groups.

These results will be discussed as they relate to current theories of hippocampal function.

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11.5 SEVERE RECOGNITION IMPAIRMENT AFTER COMBINED BUT NOT SEPARATE TRANSECTION OF THE FORNIX AND THE AMYGDALOFUGAL PATHWAYS. J.Bachevalier*, J.K.Parkinson, J.P.Aggleton and M.Mishkin (SPON: K.Macko). Lab. Neuropsychology, NIMH, Bethesda, MD 20205.

Two relatively independent limbo-diencephalic systems appear to contribute equally to recognition memory in the monkey (Mishkin and Saunders. Neurosci.Abstr. 5:1979). One is a hippocampo-diencephalic system interconnected by the fornix (Fx) and the other is an amygdalo-diencephalic system interconnected by amygdalofugal pathways (AFP) including both the ventral amygdalofugal pathway and the stria terminalis. To test further the postulated equivalence of these two parallel systems in recognition memory, we examined the effect of limbo-diencephalic disconnection on monkeys' visual memory by transecting the two pathways either alone or in combination.

Twelve cynomolgus monkeys were trained preoperatively in a one-trial recognition task in which they were required to distinguish a completely novel object from a sample object presented 10 sec previously (delayed nonmatching-to-sample). After achieving the criterion of 90 correct responses in 100 trials, three monkeys each underwent bilateral transection of either Fx, AFP, both, or neither. Fx transections were made by aspiration through a slit in the corpus callosum, while AFP transections were made by aspiration through dorsal amygdaloid tissue via an orbitofrontal approach. Following reattainment of criterion, the animals were given a performance test with longer delays (30 to 120 sec) and list lengths (3 to 10 objects). Whereas scores of the normal monkeys on this performance test ranged from 89 to 93%, and those of the monkeys with Fx or AFP transections were only slightly lower (range: 84-93% and 84-94%, respectively), the mean performance score of monkeys with combined Fx and AFP transections fell sharply (range: 69-74%). Histological examination indicated that the AFP lesions resulted in ischemic damage to part of the amygdaloid complex, and thus they cannot be characterized as transections only. Nevertheless, the additional damage to the amygdala yielded an impairment no more severe than that seen after Fx transection and was not sufficient by itself (AFP group) to produce the substantial memory deficit observed when a Fx transection was superimposed (Fx + AFP group).

The results suggest that disconnection of both the amygdala and the hippocampus from the diencephalon by combined transection of their diencephalic connections results in a memory impairment similar to (though less severe than) that following combined damage to the limbic structures themselves. The data thus provide additional evidence for the complementary participation of amygdalo-diencephalic and hippocampo-diencephalic circuits in recognition memory.

11.6 AMYGDALECTOMY BUT NOT HIPPOCAMPECTOMY IMPAIRS CROSS-MODAL DELAYED NONMATCHING-TO-SAMPLE IN MONKEYS, E. A. Murray and M. Mishkin. Lab. of Neuropsychology, NIMH, Bethesda, MD 20205.

After extensive pre- and post-operative testing on delayed nonmatching-to-sample (DNMS) in the tactual modality and, separately, in the visual modality, cynomolgus monkeys with ablations of either the amygdaloid complex or the hippocampal formation were tested on cross-modal DNMS. In the cross-modal task the animals were required first to touch and displace in the dark a sample object overlying the baited central well of a three-well testing board (sample presentation). Then, 10s later, the monkeys were presented with the sample object plus another one overlying the two lateral wells, but now in the light (test); the animals could obtain a reward by displacing the object that had not been touched 10s before, but they had to select it visually because a response was scored the moment either object was first touched. The animals were tested with a fixed set of 40 tactually distinctive objects that were randomly paired daily. These were the same objects that had been used in the pre- and post-operative intramodal DNMS testing, both in the light and in the dark (Murray and Mishkin, Neurosci. Abstr., 7: 237, 1981).

Although both groups of animals had previously achieved a high level of performance on each of the intramodal DNMS tasks with 10s delays between sample presentation and test (amygdalectomy: 90% and 98% correct responses in touch and in vision, respectively; hippocampectomy: 92% and 99% correct responses in touch and in vision, respectively), the performance of the two groups differed markedly on the cross-modal DNMS task. Monkeys with bilateral hippocampectomy performed well on the tactual to visual DNMS, averaging 88% correct responses across 500 trials. In contrast, monkeys with bilateral amygdalectomy averaged only 57% correct responses across the 500 trials. For both these groups there was little improvement with training; monkeys performed at about the same level over the last block of 100 trials as they had over the first block. These results suggest that amygdalectomized but not hippocampectomized monkeys are impaired in either (i) the ability to form an association between stimuli of different sensory modalities even though they are derived from the same external object or (ii) the ability to recall an object in one sensory modality given an input or trigger stimulus in another modality. These findings have broad theoretical import regarding the role of the amygdaloid complex in associative memory. Although it had been shown earlier that the amygdala is important for either forming or recalling object-reward associations (Speigler and Mishkin, Behav. Brain Res., 3: 303, 1981), the present results suggest that in fact the amygdala may be critical for the formation or recall of sensory-sensory associations in general.

11.7 A SELECTIVE MNEMONIC ROLE FOR THE HIPPOCAMPUS IN MONKEYS: MEMORY FOR THE LOCATION OF OBJECTS, J. K. Parkinson* and M. Mishkin. Lab. of Neuropsychology, NIMH, Bethesda, MD. 20205.

Six cynomolgus monkeys were trained by approximation to remember the particular locations occupied by particular objects on the basis of a single acquisition trial. Testing was conducted with trial-unique objects and a three-well stimulus tray in a Wisconsin General Testing Apparatus. In the final phase of preoperative testing, two different unbaited sample objects were presented on two wells selected at random, and the animal was required to displace both objects in order to receive a baited test trial 12 seconds later. On this test trial, one of the two previously presented samples was presented again on the well it had covered in the acquisition trial, while an exact duplicate of this object was presented either (a) on the other well that had been covered during acquisition or (b) on the third, previously uncovered, well. Choice of the sample object in its original position was rewarded. In the case of test trial (b) correct choice required memory of location only, whereas in test trial (a) it required memory of both the object and its location. There were 12 possible combinations of stimulus settings across acquisition and test trials, and each combination was presented twice daily for a total of 24 test trials per day.

After 25 days of testing on this final design, the six animals were divided into two groups of three matched on the basis of their preoperative performance and given bilateral ablations of either the amygdaloid complex or the hippocampal formation. Ten days postoperatively, the animals were retested on the final design of the task. The amygdalectomized monkeys reattained their preoperative performance levels of about 80% correct responses within 25-40 sessions. By contrast, the hippocampectomized monkeys continued to perform at chance levels through 75 sessions, the maximum presented. The scores for test trials (a) and (b) were about the same for a given animal at a given stage, suggesting that under the conditions of this experiment the animals treated the object and its location as a unit.

The present results contrast with previous findings both on object recognition, where hippocampal and amygdaloid lesions were found to yield equivalent though mild effects, and on object-reward association, where amygdaloid lesions were found to yield more severe effects than those of hippocampal lesions. The overall pattern of results suggests that the hippocampus in the monkey contributes selectively to the rapid memorization of the locations of objects.

11.8 SPATIAL MEMORY AND LONG-TERM RETENTION FOLLOWING MAMMILLARY BODY LESIONS IN MONKEYS. E.J. Holmes, S. Jacobson, B.M. Stein* and N. Butters. Lab. for Neurosurg. Neurobehav. Res., Boston VA Medical Ctr., Boston, MA 02130.

Although most of the experimental evidence indicates that surgical destruction or disconnexion of the mammillary nuclei does not affect the retention of information acquired before surgery, the dispute as to whether the mammillary nuclei are or are not key structures involved in the short-term retention of new information remains, for the most part, unsettled. It also remains unclear as to whether subjects with mammillary body (MB) lesions are more prone than intact control subjects to a retention loss of the solution to a previously acquired task following a prolonged period of time without practice. In the following study, we attempted to at least partially resolve these two questions.

The subjects were nine cynomolgus monkeys (M. fasicularis), all of which were equal in their previous training experience. Using a direct sub-temporal approach to the hypothalamus, three subjects had previously received bilateral lesions of the mammillary bodies, three subjects received surgical control lesions for the sub-temporal approach, and three subjects remained unoperated. In a previous experiment, all of the subjects had been trained on a delayed alternation (DA) task in which they were required to alternate responses between left and right reward sites from trial to trial with a 5 second delay between trials. The subjects were trained on the DA task for 50 trials per day until they reached a learning criterion of 90 correct responses in 100 consecutive trials. Approximately one year after initial training, the subjects were again trained to criterion on the DA problem as before, after which the demand on short-term memory in the task was increased by inserting a delay of 30 secs. between trials in place of the previous 5 secs.

In the first part of the experiment, all three groups were able to reattain criterion performance on the 5 sec. DA task with relative ease. That is, even after a time lapse of 12 months, the subjects with mammillary body (MB) lesions did not differ significantly from either the intact or operated control animals in relearning the original DA task. In the second part of the experiment, however, the subjects with MB lesions showed marked difficulty in attaining the new DA task which utilized a 30 sec. intertrial interval. Moreover, the performance of the MB lesion group was in marked contrast to the two control groups which displayed little or no difficulty in adapting to the increased delay. Hence, the results indicate that the mammillary nuclei in nonhuman primates play a significant role in the ability to retain or process information in short-term spatial memory, although these nuclei do not appear to be involved in the long-term retention of a previously acquired spatial skill.

11.9 TWO FORMS OF AMNESIA IN MONKEYS: RAPID FORGETTING AFTER MEDIAL TEMPORAL LESIONS BUT NOT DIENCEPHALIC LESIONS. S. Zola-Morgan,* and L.R. Squire. (SPON: R. Loy). VA Medical Center, San Diego, CA 92161 and Dept. of Psychiatry, UCSD Sch. of Med., La Jolla, CA 92093.

It has been known for a long time that amnesia can occur after damage in two different brain regions, the diencephalon and the medial temporal region. Recently, it has been suggested that diencephalic and bitemporal amnesia might be fundamentally different entities (Huppert and Piercy, 1978; 1979; Squire, 1981). These studies used a method designed to minimize the difficulty of comparing forgetting rates between normal and amnesic groups that already differ in level of acquisition, and demonstrated that amnesic patients with diencephalic damage exhibited a normal rate of forgetting, while those with bitemporal dysfunction exhibited an abnormal rate of forgetting. These results support a scheme for classifying amnesia based on behavior and the presumed underlying neuropathology. However, no neuropathological information for any of the amnesic cases is available. Studies using monkeys with circumscribed lesions of the critical brain areas could provide direct evidence for an association between nature of amnesia and site of lesion.

Monkeys with bilateral conjoint removal of the hippocampus and amygdala (A+H=4), those with bilateral medial thalamic removal including dorsomedial nucleus (DM=4), and a group of normal control monkeys (N=3) were first trained on the delayed non-matching-to-sample task, which is sensitive to amnesia in humans. On this task all groups received only single exposures to the sample stimulus. Next, following a procedure analogous to that used in the human studies, operated monkeys received repeated exposures to the sample stimulus in order to equate their retention performance to that of normal monkeys at 10 min after training (A+H: 12 exposures; DM: 10 exposures; N: 1 exposure). Forgetting was then assessed at 1 hr and 24 hr after acquisition. Our findings paralleled those observed in human amnesia. While both operated groups were impaired in the single exposure condition, in the multiple exposure condition monkeys with A+H lesions exhibited rapid forgetting while the monkeys with DM lesions exhibited normal forgetting rates. The results confirm the hypothesis that medial temporal and diencephalic brain regions normally contribute in different ways to the formation of memory. The significance of these findings to the development of an animal model of amnesia will be considered.

11.10 COMPARISONS AMONG FORMS OF AMNESIA: SOME DEFICITS ARE UNIQUE TO KORSAKOFF SYNDROME. L.R. Squire, N.J. Cohen,* and S. Zola-Morgan,* VA Medical Center, San Diego, CA 92161, Dept. of Psychiatry, UCSD Sch. of Med., La Jolla, CA 92093 and Dept. of Psychology, MIT, Cambridge, MA 02139

Certain features of abnormal memory, which have figured prominently in theoretical treatments of the amnesic syndrome, were assessed in patients with Korsakoff syndrome, case N.A., and patients receiving electroconvulsive therapy (ECT). Patients with Korsakoff syndrome differed from the other patients by 1) failing to exhibit release from proactive interference (PI); and 2) being disproportionately impaired in the ability to make judgments about the temporal order of recent events.

A number of considerations suggested that these deficits might be related to frontal lobe damage, and be superimposed upon a more basic memory disorder. To explore this possibility, three tests sensitive to frontal lobe dysfunction (card-sorting, word fluency, and an embedded figures test) were given to the Korsakoff patients and to case N.A., two examples of diencephalic amnesia. Whereas N.A.'s performance on these tests was average or above-average, the Korsakoff patients were impaired. Moreover, there was a significant correlation (r=.79) between their performance on the three tests sensitive to frontal lobe dysfunction and performance on those aspects of the memory tests purported to reflect frontal lobe dysfunction (the tendency to release from P.I. and the ability to make temporal order judgments). By contrast, there was not a significant correlation (r=.14) between their performance on these three tests and performance on those parts of the memory tests that gave difficulty to all the amnesic patients (trials 1-4 of the 5-trial P.I. test and recognition memory). These results indicate that the Korsakoff syndrome is unique to some extent, in that this syndrome is associated with cognitive deficits that occur together with amnesia and that can determine its character. Presumably critical diencephalic lesions, which cause the memory disorder, occur in this syndrome together with frontal lobe dysfunction. The frontal lobe dysfunction appears to superimpose additional cognitive deficits upon the basic memory disorder. Theories of amnesia, and inferences from amnesia about the organization of memory in the brain, must distinguish between the basic memory disorder in amnesia and other deficits that have no obligatory relationship to the basic disorder.

11.11 THE EFFECT OF PERIARCUATE AND PRINCIPALIS LESIONS ON THE PERFORMANCE OF A MOTOR CONDITIONAL ASSOCIATIVE-LEARNING TASK. Michael Petrides* (SPON: B. Milner). Dept. of Psychology and the Montreal Neurological Institute, McGill Univ., Montreal, Quebec, Canada, H3A 2B4.

It has been shown that patients who have sustained unilateral excisions from the frontal cortex are severely impaired in mastering conditional associative-learning tasks (Petrides, M. cited in Milner, B. Philos. Trans. R. Soc. Lond., in press). The frontal lobe is a large and heterogeneous area as shown by a number of neuroanatomical and behavioral investigations. Determining the critical area within the frontal lobe that gives rise to these deficits is difficult in work with patients because the therapeutic excisions overlap to a considerable extent. This problem can, of course, be overcome with experimental animals. The experiment reported here investigated the effect of selective frontal-lobe lesions in the monkey on a test modelled on one of the tasks used with patients.

Ten adult rhesus monkeys (Macaca mulatta) were used. Three animals served as unoperated controls. Three animals received bilateral ablations of the cortex lying within the sulcus principalis and the cortex lying immediately above it, and four animals received bilateral ablations of the periarcuate region.

In the main experiment, the monkeys were faced with a box covering the foodwell. The box had two manipulanda attached to it: a stick the monkey could grip and a button he could touch. After mastering these two movements, the monkeys were required to learn to perform one of the movements when object A was shown and to perform the other movement in the presence of object B. The objects were shown behind the test-board and outside the monkey's reach (at a distance of 26 cm). The periarcuate monkeys were severely impaired in learning to select the correct movement in response to the appropriate stimulus. In contrast, animals with lesions to the sulcus principalis region showed only a mild retardation in learning the task.

In a control task, the monkeys were trained to respond by gripping the stick when one object was shown but not to respond to the other object. There were no differences between the groups on this and various other control tasks requiring discrimination between stimuli similar to the ones used in the main experiment. In addition, the monkeys had learned the two movements before testing began and continued to perform both movements equally well throughout testing. These facts suggest that the deficit of the periarcuate monkeys cannot be reduced to a difficulty in discriminating between the stimuli or producing the responses. It appears, instead, to reflect an inability to learn to select consistently the correct movement in the presence of the appropriate stimulus.

11.12 PICTURE ENCODING IN THE AMNESIC PATIENT H.M. Elizabeth Grove,* Mary C. Potter,* and Suzanne Corkin (SPON: W.J.H. Nauta). Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139

Although it has been suggested that amnesic patients are deficient in the encoding of new information, their capacity to perform deeper level processing has been tested only with verbal material (Butters and Cermak, 1980; Corkin, 1982). Thus, it is not known whether memory impairment with nonverbal information is associated with an encoding deficit for that material. The present study used a procedure for assessing the conceptual processing of pictures, based on a task developed by Potter (1975,1976). This test was given to the patient H.M., who has been severely amnesic for both verbal and nonverbal material since undergoing a bilateral resection of medial temporal structures in 1953 (Scoville and Milner, 1957; Milner, Corkin and Teuber, 1968; Corkin, Sullivan, Twitchell and Grove, 1981).

H.M. and normal, age-matched control subjects were shown sequences of 16 color photographs at rates of 125, 167, and 250 msec per picture. In each sequence, subjects were asked to detect a target picture that had been specified in advance by presenting either the picture itself or a brief title for the picture (e.g., "people dancing"). In the picture-target condition, the viewer could select the target on the basis of physical features alone. In the name-target condition, detection depended on identifying the subject of each rapidly presented picture, in order to match it with the title. Each picture could be processed only while it was in view, since it was subject to visual and conceptual masking by the next picture in the sequence (Potter, 1976). H.M.'s ability to detect target pictures did not differ significantly from that of control subjects in either target condition, or at any rate of presentation. His normal performance with named targets indicated that he could process pictures at a conceptual level as rapidly as normal subjects his age. A second experiment compared the performance of H.M. and control subjects on a test of recognition memory. Two sequences of 16 pictures were presented at 500 msec per picture, a rate at which it is likely that all pictures were processed conceptually. A two-alternative forced-choice recognition test was administered immediately after the presentation of each sequence. Although control subjects recognized over 80% of the pictures, H.M.'s performance was at chance.

These results suggest that a severe impairment in memory for pictures can coexist with a normal capacity for processing pictures at a conceptual level. Experiments in progress are investigating possible distinctions between H.M. and patients with Korsakoff's syndrome in the encoding of new visual information.

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11.13 CHRONIC GLOBAL AMNESIA AFTER RUPTURED ANEURYSMS OF THE ANTERIOR COMMUNICATING ARTERY. Neal J. Cohen* and Suzanne Corkin (SPON: William H. Sweet). Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139

Chronic global amnesia occurs as a result of lesions in either of two brain regions and possibly others: the medial portion of the temporal lobe, as seen in cases of encephalitis, anoxic encephalopathy, posterior cerebral artery occlusion, and surgical removal (i.e., the noted patient, H.M.); and the diencephalic midline, as seen in cases of Korsakoff's syndrome and third ventricle tumor. Although amnesias differing in etiology have traditionally been thought to comprise a single amnesic syndrome, careful neuropsychological assessment in recent years has demonstrated important differences in the severity and nature of memory impairments and in the presence of non-mnemonic deficits. An examination of the anatomical findings in relation to behavioral symptoms in patients with global amnesia enriches our understanding of the neurology and taxonomy of memory and memory disorders. Mild global amnesias sometimes accompany ruptured aneurysms of the anterior communicating artery. These amnesias are not well understood with respect to either their anatomical or behavioral correlates. In the present paper, we report preliminary findings on the status of cognition and affect in a group of such patients.

The group included 6 men, aged 48 to 61, and 1 woman, aged 37, who were examined 4 months to 18 years after rupture of an anterior communicating artery aneurysm. Neurological examinations revealed no deficits in 5 of the 7 patients. Of the two remaining patients, one had grasp reflexes bilaterally, and the other had a slight droop on the left side of the mouth. A review of the medical histories and psychiatric evaluations suggested personality change and loss of motivation following operation. The assessment of non-mnemonic cognitive functions showed a sparing of overall intelligence (Full Scale I.Q. range: 104-130), but an impairment of cognitive functions of the frontal lobes, including problem solving and verbal recency-discrimination. Memory-test results verified the clinical observations of a mild global amnesia. Measures of remote memory capacities indicated a brief retrograde amnesia. Tests of the ability to learn new material revealed deficits in recall or recognition of both verbal and nonverbal stimulus items after a delay, including memory for wordlists, prose passages, recurring verbal and nonverbal material, and complex geometric shapes. By contrast, capacities for perceptual and cognitive skill learning were intact. These findings contribute to our understanding of the patterns of deficit that characterize and differentiate amnesic syndromes.

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12.1 THE FATE OF HOST AXONS IN NERVE ALLOGRAFTS AFTER ABOLISHING TOLERANCE IN RATS. A.A. Zalewski. Laboratory of Neurochemistry, NINCDS, NIH, Bethesda, MD 20205.

Nerve allografts (grafts between genetically different animals of the same species) are rejected by normal rats but not by rats that have been rendered immunologically tolerant to donor histocompatibility antigens. Since in such tolerant animals allogeneic Schwann cells survive and myelinate host axons, it was of interest to abolish tolerance and determine what would happen to the host axons when the allogeneic cells supporting them were now rejected. Inbred Lewis (LE) and Brown Norway (BN) rats which exhibit major and minor tissue histoincompatibilities were used. Normal LE and LE rats tolerant to BN antigens received 4 cm long BN nerve grafts. Normal rats rejected their allografts whereas the entire 4 cm allograft survived at three months in tolerant hosts. The allografts in tolerant animals had numerous host axons throughout, and they were myelinated presumedly by allogeneic Schwann cells. Tolerance was abolished (by the inoculation of LE lymphoid cells that were sensitized to BN antigens) in rats that had bilateral BN nerve grafts in residence for three months. Examination of one BN graft from twelve different hosts, four weeks after the immune cell transfer, revealed that the BN graft was rejected. All these grafts were virtually acellular and lacked axons except in their proximal portion where it appeared that the host axons were attempting to regrow through the connective tissue remnants of the rejected allograft. The extent of this second host axonal growth was investigated in the other BN nerve after five more months, and it was found that regeneration proceeded only for 1-1.5 cm. The distal portion of the rejected nerve apparently underwent autolysis and disappeared. It is concluded that viable allogeneic cells (Schwann, fibroblast, vascular) together with their connective tissue matrix provided the best way to aid host nerve fiber regeneration through a long nerve allograft.

12.2 EFFECT OF A NERVE CONDITIONING LESION ON THE RATE OF NEWT LIMB REGENERATION. C. E. Maier* and I. G. McQuarrie. Dept. of Anatomy, Sch. of Med., Case Western Reserve University, Cleveland, OH 44106.

Forelimb regeneration in the newt is a nerve-dependent phenomenon. We have employed a nerve conditioning lesion (CL) in an attempt to accelerate limb regeneration by accelerating nerve regeneration. The CL effect is obtained by making an axotomizing nerve lesion, waiting 2 weeks, and making a second or testing lesion (TL) proximal to the CL. This results in an earlier appearance of regenerating axonal sprouts and an increase in their rate of elongation compared to that seen after a TL alone (McQuarrie & Grafstein, Brain Res., 216: 253, 1981).

A CL was made on the 3 major nerves of one forelimb at the elbow joint. Two weeks later, bilateral TLs were made by amputating through the mid-humerus. Daily single-blind observations of experimental and control limbs were made to record the progress of regrowth as determined by an established staging system (Singer, Quart. Rev. Biol., 27: 169, 1952). (To ensure that the contralateral control was appropriate, contralateral sham CLs were employed in some groups.) Our results showed that limb buds on the CL side completed the earliest ("accumulation") phase of regeneration approximately 4 days (15-20%) sooner than contralateral controls, apparently because limb bud formation was initiated 4 days sooner than normal.

The extent of reinnervation in early limb buds was determined 2 weeks after amputation by counting silver-stained axons in serial cross-sections of the forelimb. The number of axons per $100\mu m^2$ of limb cross-section was determined at 750μm proximal (parent axons) and 250μm distal (daughter axons) to the amputation site. In a pilot study, the number of axons per $100\mu m^2 \pm$ SD was 5.14 ± 1.15 (daughter) and 4.70 ± 1.76 (parent) on the control side, and 12.80 ± 4.53 (daughter) and 4.67 ± 1.62 (parent) on the CL side. Thus, the ratio of daughter to parent axons was 2.5X greater when the amputation was preceded by a CL. This indicates that reinnervation of the limb bud occurs more rapidly if the neurons that were axotomized by amputation had been conditioned for growth. Thus, the threshold level of innervation that is required for the initiation of limb bud formation (Singer, Rzehak and Maier, J. Exp. Zool., 166: 89, 1967) would be reached sooner, accounting for the earlier onset of limb bud formation that we observed.

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12.3 OBLIGATORY ROLE FOR POLYAMINES IN THE RESPONSE OF SYMPATHETIC NEURONS TO AXOTOMY. G.M. Gilad, Isotope Department, Weizmann Institute of Science, Rehovot, Israel

Recently, we have demonstrated that ornithine decarboxylase (ODC) activity - an indicator for polyamine biosynthesis - is rapidly enhanced within nerve cell bodies in the superior cervical ganglion (SCG) of adult rats after axotomy. This is the earliest biochemical change so far detected during the axon reaction. Presently we sought to determine: (a) whether the ODC response is specifically associated with the axon reaction or is it a nonspecific change associated with metabolic alterations of the neuron in general, and (b) whether the ODC response is an absolute requirement for the axon reaction to progress. In one set of experiments ODC activity was determined in the SCG 6h after: 1) postganglionic nerve cut (axotomy); 2) preganglionic nerve cut (denervation); 3) end organ removal, and 4) 6-hydroxydopamine treatment. The activity compared to controls was increased after all treatments by 310, 280, 150 and 170 percent respectively. In another set of experiments polyamine biosynthesis was specifically inhibited prior to, during and for 5d after axotomy was performed. When examined thereafter, this treatment resulted in an inhibition of the chromatolytic response and death of the injured neurons, while neurons of unlesioned ganglia remained intact. We conclude that in adult rats: (a) Increased ODC activity is a common feature of drastic neuronal metabolic alterations, and (b) Increased polyamine biosynthesis is obligatory for the progression of the axon reaction in SCG neurons.

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12.4 PERIPHERAL NERVE REPAIR WITH DISTRIBUTED MECHANICAL SUPPORT. L. de Medinaceli and W. J. Freed* (SPON: R.J. Wyatt)*. Div. of Special Ment. Res., NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

The introduction of microsurgical techniques has improved peripheral nerve repair in terms of the precision of fascicular reunion. Nevertheless, histological observation of longitudinal sections made shortly after repair show a profound disorganization of axons directions at the reunion site. The gap itself is usually quite wide and contains extraneous material. The present study was made to test the possibility of improving the quality of sciatic nerve repair at the axonal level in animals through the use of distributed mechanical support. A method was developed in which transverse stresses were relieved by placing the nerve in a grooved chamber, and longitudinal stresses were relieved by suturing the nerve to a rubber sleeve. Histological studies of silver-impregnated specimens from rabbit sciatic nerve using this technique revealed that the tips of the axons were closely reconnected with minimal disruption in their direction or alignment and without extraneous material between the severed stumps. In the cases with the best histological appearance, transient electrical potentials could be evoked in the distal stump by stimulation of the proximal stump. These electrophysiological phenomena were further investigated in rat sciatic nerves by similar methods. The table below shows the relationship between the histological quality of repair and the appearance of stimulus-evoked potentials in the distal stump of the severed nerve.

HISTOLOGICAL CHARACTERISTICS OF NERVE RECONNECTION

Repair Type	Apposition of closest axon tips (means ± S.E.M.)	Intervening material in gap
Reconnection, transmission of potentials	13 ± 41 microns (n=7)	0
Reconnection, no transmission	141 ± 130 microns (n=9)	+
Suture, no transmission	380 microns 320 microns (n=2)	+++

It is suggested that improved mechanical support may lead to improvements in the histological manifestations of peripheral nerve repair, and that electrophysiological studies such as these may be used experimentally to obtain an immediate evaluation of the quality of peripheral nerve repair methods.

12.5 NODAL SODIUM CHANNEL NUMBER IN NORMAL AND REGENERATED NERVE FIBERS FROM VENTRAL LUMBOSACRAL SPINAL ROOTS OF CAT. H.W. Querfurth*, R.M. Herndon, and R.A. Armstrong* Center for Brain Research, University of Rochester, Rochester, NY 14642.

Sodium channel estimates for mammalian (rabbit) peripheral nerve nodes of Ranvier as determined by ^{3}H-STX binding (7.2×10^5 sites per node (Ritchie, Rogart PNAS 74:211, 1977)) and by gating current studies (upper limit 8.2×10^4 channels/node (Chiu, J. Physiol. 309, p. 499, 1980)) are discordant by an order of magnitude. To further elucidate this problem we studied binding of ^{3}H-STX to feline lumbosacral ventral root. The parameters from a computer-fitted binding curve for 2 normal cats, were b = 1.5, B_{max} = 10.6 ± 0.6 fmoles/mg-wet, K_d = 0.94 ± .25 nM. A similar determination on 7th lumbar ventral roots from of 2 other normal cats, yielded consistent results (b = 1.5, B_{max} = 11.5 ± 1.3 fmole/mg-wet, K_d = 1.5 ± .43nM).

To calculate the nodal number per mg. of ventral root, total fibre counts and diameter histograms were prepared for spinal levels L5, L6 and L7 and several hundred internodal lengths were measured from fibres of different sizes teased from each of the lumbosacral levels. From 2 cats, there were 4372 ± 704 nodes/mg wet for L5, 4729 ± 792 for L6 and 4018 ± 153 for L7. Using the weighted mean and given B_{max}, we calculate 1.5×10^6 STX receptors per node.

Five adult cats underwent cryogenic axotomy of ventral root levels, L5, L6, L7 and S1 on the left side. After regeneration for 16-45 weeks, binding parameters were determined. On the right (control) side binding was consistent with that in unoperated animals (b = 1.3, B_{max} = 10.2 ± .4 fmol/mg-wet, K_d = 0.6 ± .1 nM). However, the regenerated nerves showed a 3.6x increase in maximal binding (b = 1.3, B_{max} = 36.1 ± .5, K_d = .45 ± .04). Computer aided histologic analysis of the regenerated roots for 1 cat (19 weeks) revealed 1) a decrease in fibre size; 2) a significant decrease in internodal length for fibres in a given size class; 3) a 1.5x increase in total fibre count per root. These factors account for a 3.1x increase in nodes per mg wet. The number of STX sites per regenerated node was calculated to be 1.7×10^6.

It is concluded that 1) a very high number of STX receptors exists at the node of Ranvier of mammals; 2) Anterior horn cells possess the capability of synthesizing STX binding protein in response to injury; 3) the number of channels/node remains relatively constant in the regenerated fibers. Channel density remains unclear since effective nodal area and channel size are not as yet clearly known. A similar increase (2.7x) in channel number per unit length was seen in remyelinated peripheral nerve in rabbit (Ritchie, Rang, Pellegrino, Nature 294:257, 1981).

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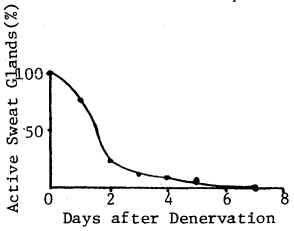
12.6 DENERVATION AND REINNERVATION OF SWEAT GLAND. W.R.Kennedy, M.Sakuta* Dept.of Neurology, Univ.of Minnesota, Minneapolis, MN 55455

PURPOSE We report the response of denervated mouse sweat glands (SG) to daily S.C. pilocarpine and the progress of their re-innervation by the saphenous nerve.

METHODS Two groups of animals were studied. In Group I, the sciatic and saphenous nerves were sectioned. In Group II only the sciatic nerve was sectioned. S.G. were activated by nerve stimulation or S.C. pilocarpine. Sweating was evaluated using a silicone elastic impression material that detected the number and distribution of active S.G.

RESULTS In the intact mouse electrical stimulation of the sciatic nerve activated all S.G. in the hind paw. Saphenous nerve stimulation activated a few S.G. on digits 1 and 2. Pilocarpine (SC) activated 90% of innervated S.G. In Group I the number of S.G. activated by pilocarpine declined daily to zero by day 7 (fig.). The latency from injection to onset of sweating progressively lengthened. In Group II by 2 to 3 weeks the number of active glands in the saphenous territory had increased and the territory enlarged far outside the original confines to other foot pads and digits. The spreading reinnervation ceased after 6-8 weeks. That the saphenous was the source of axons to the newly reinnervated S.G. was proven by their response to electrical stimulation of the nerve and by their subsequent failure to respond to pilocarpine one week after the saphenous nerve was sectioned. In animals where the sciatic nerve was allowed to regrow into the paw the extent of the collateral reinnervation of the saphenous nerve was inhibited.

CONCLUSIONS Mouse sweat glands do not develop denervation hypersensitivity. After sciatic nerve section the remaining intact saphenous nerve collaterally reinnervates 6 to 9 times the number of glands normally innervated. This increases the density and enlarges the area of the original sweat territory. The collateral reinnervation is inhibited by reappearance of the tibial nerve. The remarkable degree of regeneration by collateral sprouting is considered in relationship to the sprouting patterns of other sensory and motor axons.



12.7 REGENERATION AND MATURATION OF MAMMALIAN MYELINATED AXONS: INTRA-AXONAL STUDIES. J.D. Kocsis, S.G. Waxman, and C. Hildebrand*. Dept. of Neurology, Stanford Sch. of Med. and V.A. Med. Ctr., Palo Alto, CA. 94304; Dept. of Anat., Karolinska Inst., Stockholm, Sweden.

Several studies indicate that voltage-dependent potassium conductances (g_K) may be absent or minimal at mammalian nodes of Ranvier, but are present at internodal and on nonmyelinated axons. We studied the effects of the g_K blocking agent 4-aminopyridine (4-AP) on the waveform and firing properties of intra-axonally recorded action potentials from regenerating rat sciatic nerve, and compared these results to those obtained from myelinated fibers of young adult and mature rats.

Wistar rats were anesthetized with pentobarbital and their sciatic nerves crushed for 20 sec with fine forceps. The regenerating nerves were removed, desheathed, and placed in a recording chamber into which warmed (37 deg C) Ringer was continuously applied. The proximal end of the nerve was stimulated and the whole nerve response recorded at various points along the nerve. Intra-axonal impalements were obtained with glass microelectrodes filled with 2M KCL. The nerves were also studied ultrastructurally.

Early regenerating fibers (3 to 14 days) had conduction velocities ranging from 1-3 m/sec. These fibers were identified ultrastructurally as premyelinated and gave rise to numerous axonal sprouts extending into Schwann cell sheaths of the distal nerve segment. The predominant effect of 4-AP on these fibers was broadening of the action potential. After 3 mo of regeneration most of the fibers in the distal segment of the sciatic nerve were myelinated. Conduction velocities of these regenerated myelinated axons were similar to normal sciatic nerve (30-50 m/sec). When 4-AP was applied to the myelinated regenerated axons, the whole nerve response developed late negative components that gave a "rippled" appearance. Single axon recordings indicate that spike bursting occurred following a single stimulus, suggesting that the damped oscillations in the whole nerve response were due to repetitive firing, rather than to the addition of new fiber activity or a simple delay in repolarization. In mature rats (3 mo or more) 4-AP had virtually no effect on the myelinated component of the nerve response or on the waveform of single action potentials. But, when 4-AP was applied to myelinated fibers of young adult rats, the effects of 4-AP were similar to those observed for myelinated regenerating fibers, i.e., the elicitation of bursting.

These results indicate that the functional organization of regenerating myelinated axons is similar to that of myelinated axons of young adult rats, but differs from myelinated axons of mature rats.

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12.8 REGENERATION OF PRESYNAPTIC ACTIVE ZONES AT THE FROG NEUROMUSCULAR JUNCTION. Chien-Ping Ko. Dept. of Biological Sciences. University of Southern California, Los Angeles, CA 90007

The active zone of the nerve terminal has a unique structure and is thought to be the site of transmitter release. However, it is not clear how this unique specialization is formed and at what stage in its morphogenesis, the active zone becomes functional. To answer these questions, reinnervation of neuromuscular junctions in the frog cutaneous pectoris muscle was studied by combining intracellular recording and freeze-fracture electron microscopy.

Around 11 days following nerve crush, reinnervation began. The percentage of junctions which showed evoked endplate potentials increased over a period of one week. During this period, some terminals were seen to make small synaptic contacts in the middle of the width of the gutters, which were otherwise occupied by Schwann cells. At these newly regenerated contacts, there were clusters of large intramembrane particles located just opposite to junctional folds. Some of these clusters already showed the typical orientation and two-double-row organization found at normal terminals. However, these primitive active zones were very short. If nerves were stimulated in dilute formaldehyde, dimples, which may represent openings of synaptic vesicles, were seen at these primitive active zones and even at regions of terminal membrane where there were only clusters of unorganized particles opposed to junctional folds.

Around 20 days post-denervation, every muscle fiber recorded had endplate potentials. Nerve terminals became larger and occupied most of the area of the junctional gutters. However, active zones were still not completely regenerated even at one month following denervation. Often there was more than one short segment of primitive active zones opposed to one junctional fold. Openings of synaptic vesicles were found along both sides of these discontinuous active zones, but were not found in the regions where active zones were interrupted. Later, these discontinuous short segments of active zone probably joined together and formed the normal mature active zone. About three months after nerve crush most active zones were indistinguishable from the normal.

This study suggests that transmitter release can occur even before active zone particles are organized into the adult pattern of two double rows and localization of the presynaptic active zone may be induced by the junctional fold.

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12.9 EFFECT OF PRIOR COLLATERAL SPROUTING ON AXONAL REGENERATION. Janet R. Sparrow and Bernice Grafstein. Dept. Physiology, Cornell Univ. Med. College, New York, N.Y. 10021

It has repeatedly been shown that a faster rate of axonal regeneration occurs after the second of two nerve injuries (McQuarrie and Grafstein, Arch Neurol. 29:53, 1973; McQuarrie, Brain Res. 152:597, 1978). However it is not known whether the earlier "conditioning lesion" is effective because of injury to the axons per se or whether it is related to the axonal outgrowth which occurs after the first lesion. We have undertaken to induce prior outgrowth in the absence of a lesion and to determine whether an enhancing effect on subsequent axonal regeneration occurs.

Partial denervation of the muscles in the right hind limbs of young rats was performed by eliminating L4 and L6 spinal nerves. Control rats received a sham operation. Collateral sprouting of L5 axons was allowed to proceed for 7 days postoperatively, upon which the right sciatic nerve was crushed. The distance regenerated by the axons 8 days after crushing was measured by labeling the motor axons with radioactive fast transported proteins. ^{3}H-proline was injected into the lumbar ventral horns and 24 hours later the animals were killed and the sciatic nerves were removed. The radioactivity in consecutive 2 mm segments of nerve was measured by liquid scintillation and plotted as a function of distance from the site of crushing.

In nerves which had undergone prior sprouting the mean distance regenerated was significantly greater than in control nerves ($p < 0.01$). Conversely, the distance regenerated by the fastest growing axons (i.e. maximum outgrowth distance) was not increased.

It is concluded that prior axonal outgrowth, induced without injuring the axons, serves to accelerate the outgrowth of motor axons regenerating after a crush. The enhancement of regeneration is comparable to that induced by an earlier conditioning lesion: the mean rate of outgrowth is accelerated while the fastest growing axons are unaffected. Thus the conditioning lesion effect may be attributable at least in part to a mechanism which is associated with outgrowth and which is not dependent on axonal injury for its initiation.

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12.10 DOES NEUROTOXIC CONDITIONING ACCELERATE NERVE REGENERATION? A.B. Sterman. Department of Neurology SUNY at Stony Brook, Stony Brook, NY 11794 and Northport VA Medical Center, Northport, NY.

We have recently shown that prototype neurotoxins, putatively directed exclusively at axons, produce a spectrum of cell body remodeling resembling chromatolysis (Sterman, 1981; in press). This study tested the hypothesis that "conditioning" by exposure to a prototype neurotoxin would accelerate the rate of regeneration of severed peripheral axons by evoking a reparative reorganization of the soma, analogous to the well-studied conditioning lesion phenomenon. Thirty-eight Sprague-Dawley rats were paired, and each pair member either exposed to 0.5% 2,5-hexanedione (2,5-HD) in drinking water (experimental) or received water without toxin (control). After six weeks, the rats were given a one week recovery period off toxin and then were subjected to experimental crush of the sciatic nerve, using the general methods of others (McQuarrie, 1977; Forman, 1978). Nerve regeneration was measured by the pinch test 5-13 days after crush. Results failed to show an accelerated rate of axonal outgrowth; however, there was a small but significant ($p < 0.05$) decrease in the initial delay from sciatic nerve crush to the start of outgrowth. Thus, despite morphological similarities between neurotoxin-induced cell body changes and chromatolysis, there are critical functional differences. Results provide a model (morphologically similar to chromatolysis but functionally divergent) which can be used to explore factors supporting nerve regeneration.

This study was supported in part by the Veterans Administration, NIRA ES02650, and MH36856.

12.11 FUCOSYL GLYCOPROTEIN DISTRIBUTION IN THE DEMYELINATED PERIPHERAL NERVE. I. M. Parhad, J. W. Griffin*, D. L. Price*, J. F. Koves*, and D. O. Kuethe*. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

The effect of demyelination on axonal transport has been incompletely studied. The problem is of interest because transported glycoproteins might be involved in remodeling the axolemma in demyelinated internodes or in influencing Schwann cells to divide or remyelinate. [^{3}H] fucose, when injected near nerve cell bodies, is incorporated into fucosyl glycoproteins. Fucosyl glycoproteins are carried predominantly by fast axonal transport; a portion of these glycoproteins become associated with axolemma. The present study was designed to assess whether there was altered retention of rapidly transported fucosyl glycoproteins in demyelinated internodes.

A 2% lysolecithin solution was applied directly to a segment of the sciatic nerve of Sprague-Dawley adult rats for ten minutes. Ten to thirty percent of fibers developed demyelination, mostly in the subperineurial area. One to five days after application of lysolecithin, [^{3}H] fucose was injected into the lumbar ventral horns and dorsal root ganglia (L4-L5). The sciatic nerves were fixed with glutaraldehyde 2-7 days after labeling when most of the rapidly transported material had moved past the site of demyelination. The tissue was embedded in Epon, and longitudinal sections of nerve were processed for light autoradiography. Area measurements were done using a digitizer. Both in the normal (myelinated) and in the demyelinated fibers, there was a marked increase in grain density in the constricted segment (node and myelin sheath attachment sites) of the axon. In the demyelinated internodes (DI), there was at all times an increase in the density of silver grains as compared to the normally myelinated adjacent internodes (MI) of the same axon (DI: 0.38±0.03 grains/μm^2; MI: 0.20±0.02 grains/μm^2; 23 matched pairs examined). The demyelinated internodes had a smaller caliber when compared to the normally myelinated adjacent internodes of the same axon (DI: 3.83±0.22μm; MI: 4.45±0.31μm).

These results showed a markedly increased retention of rapidly transported fucosyl glycoproteins in the constricted segments of both the normal and demyelinated axons. In the demyelinated internodes, there was increased retention of fucosyl glycoproteins as compared to the normally myelinated internodes. The increased retention of fucosyl glycoproteins may be due to local slowing of transport and/or to increase insertion in the axolemma.

12.12 INCOMPLETE GLYCOSYLATION OF A MYELIN GLYCOPROTEIN EXPRESSED BY SCHWANN CELLS IN THE DISTAL SEGMENT OF THE PERMANENTLY TRANSECTED NERVE. Joseph F. Poduslo, Carole T. Berg*, and Peter J. Dyck. Membrane Biochemistry Laboratory, Peripheral Nerve Center, Department of Neurology, Mayo Foundation, Rochester, MN 55905.

Glycoproteins have been implicated in Schwann cell-axon recognition events responsible for the initiation of myelination and probably for the maintenance of the integrity of the myelin sheath (Poduslo, in Peripheral Neuropathy, eds. Dyck, Thomas, Lambert, and Bunge, in press). The elucidation of the regulatory mechanisms by which Schwann cells express specific myelin proteins which are then assembled into the myelin membrane is an important area of research. Schwann cell expression of the major myelin glycoprotein, P_o, has been investigated using the permanent nerve transection model described by Spencer et al (Brain Res., 165, 119, 1979) where reinnervation and remyelination is prevented. Endoneurial fractions from a desheathed nerve distal to the permanent transection were compared with similar fractions from a crushed nerve at 7, 14, 21, 28, and 35 days after injury. The expression of P_o glycoprotein was evaluated after SDS-pore gradient electrophoresis by Coomassie blue and silver stain and by autoradiography after direct overlay of radioiodinated lectins (wheat germ agglutinin, gorse agglutinin, and concanavalin A). As evaluated by these parameters, the concentration of P_o after crush decreased and subsequently increased as a function of time after injury corresponding to the events of demyelination and remyelination. After permanent transection using Coomassie blue stain (sensitivity: ~100 ng), the P_o concentration decreased following the same time course found after crush. At subsequent time points, P_o could not be detected using Coomassie blue stain which supports the observations made by Spencer (in Aspects of Developmental Neurobiology, eds. Ferrendelli and Gurvitch, 1979). Similar results were obtained when P_o was evaluated using silver stain which has a ~50 fold greater sensitivity than Coomassie blue (~2 ng). Wheat germ agglutinin (sensitivity at the subnanogram level) (specificity: NeuNAc-Gal-GlcNAc) showed no binding to P_o glycoprotein by 21 days post-transection; however, both gorse agglutinin (specificity: Fuc) and concanavalin A (specificity: Man) showed low levels of binding to P_o. Radioactive precursor incorporation studies of endoneurial slices at 35 days post-transection revealed active synthesis of P_o glycoprotein by Schwann cells in this permanent transection model. In addition, differences in lectin binding and precursor incorporation studies suggest that this glycoprotein is incompletely glycosylated since we could not demonstrate addition of the terminal carbohydrates. The data suggest a post-translational regulation step in the synthesis of this glycoprotein and the subsequent regulation of myelination.

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12.13 EFFECT OF XYLOCAINE APPLIED TO THE HYPOGLOSSAL AXOTOMY SITE ON 14C-2-DEOXYGLUCOSE UPTAKE IN THE HYPOGLOSSAL NUCLEUS. Philip A. Singer and Sharon Mehler*, Neuromuscular Histochemistry Laboratory, Veterans Administration Medical Center, Kansas City, MO 64128.

We (Singer and Mehler, Exp. Neurol. 69:617-626, 1980) and others (Kruetzberg and Emmert, Exp. Neurol. 70:712-716, 1980) have found markedly increased 2-deoxyglucose (2DG) uptake in the hypoglossal nucleus as early as 24 hours after hypoglossal axotomy. There is a question whether this increased uptake is caused by increased synthetic activity or perhaps increased numbers of action potentials. David and Aguayo (Neuroscience Abst. 6:439, 1980) have shown that increased 2DG uptake in the lumbar anterior horns is blocked by applying xylocaine to the axotomy site 13 days after the sciatic nerve was cut and placed in a polyethylene tube. Thus, presumably, action potentials were originating in a neuroma and invading the anterior horn cells. In order to test the possibility that a similar mechanism was producing the increased 2DG uptake in the hypoglossal nucleus after axotomy, the following study was undertaken.

Male 150-200gm Sprague-Dawley rats underwent bilateral hypoglossal ligation followed by axotomy distal to the ligation under nembutal anesthesia. The nerve was ligated so that the proximal cut end could be identified later. In animals destined for longer (15 days) survival, the hypoglossal was ligated and cut only on the left. At 24 hours, 3 and 15 days after the axotomy the left proximal hypoglossal stump was exposed under nembutal 50mgm/kg anesthesia and a cotton pleget soaked in 1% xylocaine applied around the nerve stump. 15μc/100gm 14C-2-deoxyglucose was then injected via the tail vein. Forty-five minutes later the brainstem was excised and frozen in freon. Later 20u sections were cut at -18° and applied to x-ray film for 2 weeks. The resultant autoradiographs showed no difference in glucose uptake between the xylocaine treated and control sides at 24 hours or 3 days and a definite increased uptake compared to the non-axotomized side at 15 days.

Our results suggest that electrical activity originating from the axotomy site is not a factor in producing increased 2DG uptake in the hypoglossal nucleus. The difference between our results and those of David and Aguayo may be explained by several differences in the preparation used. We did not encourage neuroma formation by placing the axotomized nerve into a plastic tube. Furthermore, the sciatic nerve contains a large number of sensory fibers which impinge either directly or indirectly on the anterior horn and whose reaction to injury may be different from motor fibers.

13.1 CAT SCAN MEASURES IN SCHIZOPHRENICS: CORRELATIONS BETWEEN BRAIN ATROPHY, MAO-B AND DIAGNOSTIC SUBGROUPS. A Kling, N. Kurtz*, K. Tachiki* and S. Connor*. Psychiatry Service, Vet. Admin. Med. Center, Sepulveda and UCLA Sch. of Med., Los Angeles, CA.

Recent studies have demonstrated that some schizophrenics have increased ventricular brain ratios (VBR), atrophy of the sylvian fissure and other morphological changes of the brain (Weinberger, D. et al., Arch. Gen. Psych. 36:935, 1979). Other studies have implicated MAO-B levels to be low in paranoid schizophrenia (Potkin, S.G. et al., N. Eng. J. of Med. 298:61, 1978) and high in brain atrophy associated with aging and Alzheimer's disease (Adolfson, R. et al., Life Sci. 27:1029, 1980).

This study examined specific brain changes by CAT Scan and whole blood MAO-B activity in DSM III subgroups of chronic schizophrenics.

CAT Scans of 26 chronic schizophrenics (12 residual, 12 paranoid and 2 undifferentiated) between ages 25 and 45 were measured for VBR, sylvian fissue widening, and asymmetry of the frontal lobes. MAO-B levels were obtained using a modified method of Marshall (Marshall, E.F. and Campbell, I.C., Bioch. Pharm. 26:353, 1977).

Paranoid schizophrenics were found to have significantly smaller VBRs than the non-paranoid group, 7.3±3.3 vs. 10.4±3.5 ($p<.025$). The schizophrenics were divided into two VBR groups: greater than 9 (2 s.d.above normal controls 4.75±2.0). Regression and analysis of variance between the two VBR groups, MAO-B and sylvian atrophy showed a multiple correlation of $R = .689$, $p < .01$. Age and length of illness did not account for the variance seen. Individual analyses showed that MAO-B means were higher in the greater than 9 VBR groups ($p<.05$) and that sylvian fissure atrophy was positively correlated with VBR 9 at $p < .01$. Reversed asymmetry of the frontal lobes was significantly correlated ($p < .01$) with VBR values of greater than 9 when controlled for age. The paranoid schizophrenics had significantly lower MAO-B activity when compared to the non-paranoid schizophrenics ($p < .001$) and an age matched normal control group ($p < .001$).

These data suggest that chronic residual schizophrenics have larger VBRs and increased MAO-B activity compared to age matched chronic paranoid schizophrenics. Sylvian fissure atrophy was positively correlated with increased ventricular size but not with MAO-B or diagnostic subgroups.

13.2 DEMENTIA PATHOPHYSIOLOGY: X-RAY COMPUTED TOMOGRAPHY AND DYNAMIC POSITRON EMISSION TOMOGRAPHIC STUDIES WITH 18-FLUORODEOXYGLUCOSE AND RUBIDIUM-82. R.P.Friedland, T.F.Budinger*, E.Ganz*, Y.Yano*, C.Mathis*, R.Huesman*, B.Knittel*, B.Moyer*, B.A.Thompkins-Ober* and B.Koss. Donner Laboratory, University of California, Berkeley, Ca. 94720, Dept. of Neurology, University of California, Davis and VA Medical Center, Martinez, Ca..

Positron emission tomography(PET) allows for the in vivo noninvasive regional quantitation in three dimensions of aspects of cerebral physiology in health and disease. Comprehensive neuropsychological testing, x-ray computed tomography and PET studies were performed on 16 demented subjects aged 52-82 with clinical diagnoses of Alzheimer-type dementia(N=10), multi-infarct dementia, communicating hydrocephalus, Creutzfeldt-Jakob disease and alcoholic dementia. PET studies of glucose metabolism and blood brain barrier permeability were performed on the Donner 280-crystal ring (resolution 9mm FWHM). 18-Fluorodeoxyglucose(FDG) was synthesized at the Lawrence Berkeley Laboratory using the 18-F F reaction with glucal. IV injection of FDG was followed by rapid sampling of arterialized blood using hand warming to 44 C and dynamic imaging of the head from the time of injection to 60 minutes after injection. Tomographic data were collected initially at 5 second intervals and later at longer intervals in order to calculate rate constants for transport and phosphorylation of FDG and dephosphorylation of FDG-6-Phosphate. Images taken 45 minutes after injection demonstrate an impairment in glucose utilization in the temporal cortex in the Alzheimer-type dements. One subject with clinically diagnosed Creutzfeldt-Jakob disease had a unique pattern of heterogeneous and asymmetrical radionuclide distribution throughout the brain.

Studies were also performed using generator produced rubidium-82 as an indicator of blood brain barrier integrity. No rubidium-82 uptake suggestive of increased blood brain barrier permeability was noted in 4 subjects (two Alzheimer-type, one multi-infarct and one communicating hydrocephalus).

Results of kinetic analysis of FDG uptake and metabolism will be presented and relationships between behavioral and x-ray computerized tomographic features of the dementia and metabolic parameters will be discussed.

13.3 THE PATHOGENESIS OF EARLY TRAUMATIC BRAIN DYSFUNCTION. O.R. Hubschmann and D. Kornhauser*. Neurological Surgery Section, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, 100 Bergen Street, Newark, N.J. 07103 and Veterans Administration Medical Center, East Orange, N.J. 07019.

The clinical condition of closed head injury is associated with high morbidity and mortality in patients. The processes that participate in the development of the clinical presentations at the cellular level are not well understood. Using a hypothesis that the potassium is one of the primary inducing factors in the pathogenesis of cerebral swelling following head trauma, we have studied the early changes in potassium concentration following experimental head trauma. We have studied the dynamic changes in K+ and Ca++ activity in the cortical extracellular microenvironment in 15 cats where local SAH or intracerebral hematoma was produced by injecting 0.5 cc of autologous blood. The cellular response was monitored using extracellular K+ and Ca++ ion-specific microelectrodes, DC cortical potential and electrocorticogram.

The cellular response was characterized by a profound cellular depolarization, extracellular Ca++ depletion and extracellular accumulation of K+. The pre-hemorrhagic baseline K+ level averaged 3.17 ± 0.52 mM and was elevated to levels ranging between 16 and 31 mM. Ca++, normally maintained at 1.14 ± 0.11 mM was reduced to levels ranging between 0.4 and 0.7 mM. In most experiments, both K+ and Ca++ activity returned to normal limits within 5 minutes.

Experimental head trauma, either in the form of subarachnoid hemorrhage or intracerebral hematoma, is accompanied by the release of potassium into the extracellular space accompanied by concomitant extracellular calcium depletion and depression of electrical activity. This change is not dependent on intracranial pressure and it is not induced by ischemia. The potassium release which appears to be transient, in most instances, blocks neuronal transmission and causes increase in vascular resistance. It is combined with the decrease in extracellular calcium which change the cell permeability, decrease their metabolic activity and block synaptic transmission. We conclude that this process is the pathogenetically primary force in inducing secondary brain damage in head trauma.

13.4 ULTRASTRUCTURAL STUDY OF THE ASTROCYTIC CHANGES INDUCED BY SPONTANEOUS SPONGY DEGENERATION OF THE MOUSE BRAIN. N.A. Azzam, P.A. Cancilla*, J.V. Bready* and R.N. Azzam*. Faculty of Med., Kuwait Univ. and College of Med., The Univ. of Iowa, Iowa City, IA 52242.

A spontaneous spongy degeneration was discovered in the brain of the Swiss Webster mouse of the Charles River colony. The disorder is genetically transmitted in an autosomal recessive pattern of inheritance. Paraffin sections stained with H&E revealed extensive spongy degeneration affecting mainly the white matter. Ultrastructural studies confirmed the status spongiosus and localized the abnormality to the astrocyte. Astrocytic foot processes surrounding cerebral blood vessels were distended by accumulation of fluid. This caused the dispersion of cytoplasmic organelles of the foot processes which spread to include the astrocytic cell bodies. The vascular basal lamina remained intact inspite of the vacuolization. An extensive and elaborate system of hemidesmosomes appeared between the vascular basal lamina and the astrocytic foot processes. This seemed to be a reaction to help prevent the disruption of the blood brain barrier. Arterioles and capillaries remain suspended in the vacuoles by thin strands of the cytoplasmic remains of the astrocytes. Reactive astrocytes were observed in the subependymal layer. Some had large foot processes surrounding capillaries. Their nuclei were larger and cerebriform. The nuclear envelope was thrown into marked convolutions. The karyoplasm was of different densities, with few electron-dense clumps of varying sizes irregularly dispersed. The cytoplasm contained dense bodies of varying sizes, a well developed Golgi complex and several mitochondria. The most prominent feature was the presence of membrane-bound bodies with granular densities which seemed to be transverse sections of fibrils. These were widely dispersed in the cytoplasm and between the folds of the nuclear envelope. The membrane surrounding some of these bodies was ruptured and the contents were dispersed into the cytoplasm. Some bodies seemed to have merged and coalesced with other bodies. These astrocytes were held together by gap junctions and by a ball and socket type junction. Inspite of the spongy degeneration, the vascular basal lamina remains impermeable to intravenous horseradish peroxidase. However, a 5 mm spot freeze injury applied to the brain through the intact skull disrupted the blood brain barrier. An animal model is established which regularly produces affected offspring for further investigation, aimed at gaining insight into the pathogenesis of human neurologic disorders; In particular, Canavan's spongy degeneration, which bears similar patterns of inheritance and morphological abnormality.

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13.5 TO WHAT EXTENT IS TRAUMATIC PARAPLEGIA A DEMYELINATIVE DISEASE? A. R. Blight. Depts. Neurosurg. and Physiol. & Biophys., New York Univ. Med. Ctr., New York, N.Y. 10016.

It has been assumed that the cause of chronic paralysis following mechanical injury to the spinal cord is a total or near total destruction of axons at the trauma site. Several other causes of irreversible dysfunction are possible, and prominent among these is demyelination of surviving white matter. It is essential to know the relative importance of such extra-axonal pathology in considering possible therapeutic approaches to this intractable condition.

The experimental model used was contusion of the cat spinal cord with a 20g weight dropped 20cm onto the exposed dura at T7/8. Axons surviving at the injury site were examined with light and electron microscopy and in vitro intracellular recording of conduction. Animals which remained paraplegic at 7-14 months post injury were compared with animals from the 10-20% which recover the ability to stand and walk with relatively mild deficits. All animals suffered destruction of the gray and 80-95% of the white matter at the injury site. There was an overlap between paralyzed and walking animals in the number of axons surviving in the outer 150-300 um of the ventral tracts. The proportion of large caliber axons (more than 5 um) and the thickness of their myelin sheaths was greater in the walking animals. There was a clear difference between the ability of axons in the two groups to conduct through the lesion site in vitro. Impaling axons 6-7 mm either side of the middle of the lesion in dorsal, ventral and lateral tracts, the proportion capable of action potential conduction through the lesion site was determined at 22-25°C. The proportions were 13% (of 150 axons) for paralyzed, 65% (of 134 axons) for walking chronic animals, and 70% (of 233 axons) for uninjured controls.

When axons were challenged by raising the temperature, it was found that conduction failed below 36°C in 70% of axons in the paraplegic sample, compared with 7% in control and 10% in the chronic walkers. This temperature sensitivity of conduction in the paralyzed cats is consistent with morphological signs of sustained poor myelination. The evidence currently suggests that paraplegia in the model is the combined result of a critical loss of axons and persistent demyelination. Other factors, including obvious vascular occlusion, may contribute to deficits in individual cases. More attention to the cellular basis of paraplegia may open possibilities for treatment more accessible than functional axonal regeneration. (Supported by the Steven Camhi Fund for Spinal Cord Injury Research & USPHS grant NS10164 from NINCDS).

13.6 Measurement of Myelin Basic Protein and of Anti-Basic Protein Antibodies by ELISA Utilizing Biotinylated Antibodies. L. Spatz*, L. Whitman*, M-J. Messito*, G. Nilaver, S. Ginsberg* and N. Latov* (SPON:E.A. Zimmerman). Dept. of Neurology, Columbia U., New York, N.Y. 10032.

Myelin Basic Protein (MBP) is the target antigen in Experimental Allergic Encephalomyelitis and may be important in the pathogenesis of Multiple Sclerosis. Its concentration is elevated in CSF of patients with active demyelination and anti-MBP antibodies are detected in patients with Multiple Sclerosis. We compared ELISA systems for mesuring antibodies to MBP and developed a two-site ELISA capable of mesuring as little as 0.1 ng/ml of MBP.

To measure anti-MBP antibodies, microplate wells were coated with human MBP and incubated with increasing dilutions of affinity purified rabbit anti-human MBP antibodies. Binding was measured by incubating with either peroxidase-conjugated anti-rabbit IgG or Protein A, or biotinylated anti-rabbit IgG and avidin-peroxidase. O-phenelenediamine was added as substrate and the reaction products measured at 450nm. The three assay systems were equally sensitive and capable of measuring as little as 1.5ng/ml of specific antibody.

To measure concentration of MBP, wells were coated with affinity purified rabbit anti-MBP antibodies and incubated with increasing dilutions of MBP. The bound antigen was measured by incubating with biotinylated anti-MBP antibodies and avidin-peroxidase, and then with O-phenylenediamine. Reaction products were measured at 450nm. In this assay, as little as 0.1 ng/ml of MBP could be detected. The assay is significantly more sensitive than previousely described radio-immune assays for MBP and is preferable to the competitive inhibition ELISA for detecting fragments of MBP.

13.7 CAPRINE β-MANNOSIDOSIS: NEURONAL AND MYELIN ABNORMALITIES. Kathryn L. Lovell and Margaret Z. Jones. Pathology Dept., Michigan State Univ., East Lansing, MI 48824.

Morphological changes were delineated in the central nervous system of goats affected with β-mannosidosis in order to determine the extent and distribution of neuronal and myelin abnormalities associated with this inherited glycoprotein metabolic perturbation. Coronal sections of the cerebral hemispheres, sagittal sections of the cerebellum, and transverse sections of the brainstem of 3 affected goats and 3 age-matched controls, ranging in age from 0-16 weeks, were embedded in paraffin, sectioned at 6 μm and stained with Hematoxylin and Eosin and with Luxol fast blue-Periodic acid Schiff-Holmes stains. Tissues from selected areas were embedded in Epon-Araldite, sectioned at 2 μm and stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate.

Lysosomal storage vacuoles, probably representing storage of uncleaved oligosaccharides, were present to various extents in different neuronal cell types. Cell types most severely affected included cortical pyramidal cells, hippocampal pyramidal cells, caudate neurons, and cerebellar Purkinje and Golgi II cells. Other populations, e.g. granule cells from the hippocampus and cerebellum, showed less vacuolation. The size of cytoplasmic vacuoles varied among cell types and, in some cases, vacuolation appeared more severe in the oldest animal. Homogeneous, eosinophilic axonal spheroids, like those noted in α-mannosidosis, were present throughout the white matter. They were most numerous in the cerebellum and cerebral hemispheres of the 16 week old affected goat. Ultrastructural examination revealed accumulation of dense bodies and membranous material in the spheroids.

Unlike related storage disorders, gross examination revealed severe deficiency of myelin in the cerebral and cerebellar hemispheres of all animals. In contrast, the brainstem appeared unremarkable, as noted previously for the 1 day old affected animal (Jones, et al., 1979, Soc. Neurosci. Abst., 5:513). Light and electron microscopic examination, however, showed a reduction in myelinated fibers in most white matter areas, including the brainstem.

Central nervous system lesions correlated with severe neonatal neurological deficits, characteristic of β-mannosidosis. The precise role of β-mannosidase deficiency and accumulation of oligosaccharides in the pathogenesis of the neuronal and myelin abnormalities remains to be established.

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13.8 SERIAL CHANGES IN NMR IMAGES OF ACUTE EXPERIMENTAL CEREBRAL ISCHEMIA: TIME COURSE AND EFFECTS OF DEXAMETHASONE, MORPHINE AND NALOXONE. Robert M. Levy*, David Baskin*, Leon Kaufman* and Yoshio Hosobuchi* (SPON: Nancy Lee). Department of Neurosurgery and Radiologic Imaging Laboratory, University of California, San Francisco, CA 94143.

Following unilateral carotid artery ligation, about 40% of gerbils will evolve hemispheric strokes due to insufficient collateral cerebral blood flow. Baskin and Hosobuchi (1981) have demonstrated that naloxone reverses the neurologic deficit following cerebral ischemic insults in two patients and in gerbils following carotid artery ligation. Morphine was shown to precipitate stroke symptoms in asymptomatic animals. We have employed nuclear magnetic resonance (NMR) imaging to evaluate intracerebral changes over time after unilateral carotid artery ligation and the effects on these images of dexamethasone, morphine and naloxone. Gerbils were anesthetized with pentobarbital and underwent right carotid artery coagulation and sectioning. Animals were allowed to recover from anesthesia and were then tested for neurologic deficit. Both stroked and asymptomatic animals were then scanned at time points ranging from 3 to 24 hours following surgery. Symptomatic animals were then given intraperitoneal injections of either naloxone 2 mg/kg or dexamethasone 5 mg/kg and rescanned at either 5-10 minutes or 60-90 minutes later, respectively. Asymptomatic animals were injected with 10 mg/kg morphine sulfate IP and rescanned 90 minutes later. Signal intensity, T1 and T2 times were then averaged over each hemisphere of the NMR images.

The results indicate that asymptomatic animals exhibit no differences between the occluded and control hemispheres with respect to any of the above variables. Symptomatic animals, on the other hand, demonstrate significant differences between hemispheres in both T1 and T2 times. T1 times appear to be increased by 15% until 24 hours when the T1 time on the occluded side rises to 136% of the control side. T2 times appear to rise in a linear fashion over 24 hours, at which time the ischemic hemisphere demonstrates a T2 time of about 71 msec vs. a control of 57 msec.

Neither naloxone, administered to stroked animals, nor morphine, administered to asymptomatic animals, had any effect on the NMR images obtained at any time point over the 24 hours following surgery. Dexamethasone, given to symptomatic gerbils, had no effect on the NMR images obtained at either 6-8 hours or 24 hours after surgery. Of interest is that dexamethasone appears to negatively influence 24 hour survival following this cerebral ischemic insult. Thus, cerebral ischemia following unilateral carotid artery ligation in symptomatic gerbils appears to be reflected in both T1 and T2 times, and these changes appear to increase over time within the first 24 hours after surgery. None of the drugs tested appear to effect these post-ischemic changes in NMR images.

13.9 A UNIQUE PROTEIN ASSOCIATED WITH THE SCRAPIE AGENT. D.C. Bolton*, M.P. McKinley* and S.B. Prusiner, Departments of Neurology and Biochemistry and Biophysics, University of California, San Francisco, CA 94143.

The scrapie agent causes a degenerative neurological disease of sheep and goats. One infectious unit appears to induce the synthesis of 10^9 ID_{50} (mean infectious dose) units in the brain of an infected hamster over a 120 day period. The agent has been substantially purified by a method using detergent extraction, polyethylene glycol precipitation, nuclease and protease digestion, ammonium sulfate precipitation and sedimentation into a discontinuous sucrose gradient. The specific infectivity (ID_{50} units/mg protein) of the aggregated agent in a fraction from the 25%/60% interface of the sucrose gradient was increased 100- to 1,000-fold above that found in homogenates of infected hamster brain. The agent is hydrophobic and contains a protein which is required for infectivity. Attempts to demonstrate the dependence of infectivity on a nucleic acid present within the agent have been unsuccessful. The agent is resistant to ribonucleases, deoxyribonucleases, psoralen photoadduct formation, Zn^{++} catalyzed hydrolysis and chemical modification by hydroxylamine. A new term, "prion", has been introduced to describe and identify these small proteinaceous, infectious particles which are resistant to most procedures that modify nucleic acids.

A unique protein was identified in substantially purified fractions containing the scrapie agent. This protein was observed by radiolabelling of the purified fraction with N-succinimidyl 3-(4-hydroxy, 5-[^{125}I]-iodophenyl) propionate or [^{14}C]-diethylpyrocarbonate and subsequent analysis by SDS polyacrylamide gel electrophoresis. It migrated as a diffuse band with an apparent molecular weight between 27,000 and 30,000 daltons. The protein was uniquely resistant to digestion by proteinase K prior to denaturation. This property was used to demonstrate that the protein was distinct from others of similar molecular weight found in normal brain. Heating the scrapie-associated protein to 100°C for 2 min in the presence of 1.25% SDS and 1.25% β-mercaptoethanol rendered it sensitive to digestion by several proteases. The protein was degraded by proteinase K, pronase and *Staphylococcus aureus* V-8 protease under these conditions, but was apparently resistant to digestion by α-chymotrypsin. Whether this protein is a pathological product of scrapie infection or a structural component of the agent remains to be established. In support of the latter, the protein and the infectious scrapie agent copurified. In addition, preliminary results suggested that in substantially purified fractions the amount of this protein correlated with the titer of the scrapie agent.

13.10 INFUSIONS OF TRITIATED THYMIDINE INTO THE BRAIN INCREASES SURVIVAL OF BDF MICE FOLLOWING (CNS) B-16 MELANOMA IMPLANTS. M.S. Kaplan* and R.H. Selinfreund* (SPON: W.G. Dail, Jr.). Anatomy Dept., Univ. of New Mexico, Sch. of Med., Albuquerque, NM 87131.

10^5 cells of B16 melanoma were injected directly into the brains of 41 BDF female mice. Four days later a cannula was placed just above the implanted tumor. The next day infusions of either tritiated thymidine, "cold" thymidine, sterile saline, or ^{125}IUdR were administered. Total infusions of 440 μc of tritiated thymidine or ^{125}IUdR were administered over four 36-hour periods such that: 110 μc were delivered, the animals were removed from the infusion for 36 hours, 110 μc infused for another 36 hours, etc. Animals received 440 μl (total volume) of their respective agent during the four infusions.

Mice that received infusions of tritiated thymidine had a 15% increase in survival beyond those that received infusions of sterile saline or "cold" thymidine. These results were confirmed as statistically significant ($p \leq 0.01$) by Duncan's multiple range test for variable time and by least square means. Analysis of radioactivity in individual organs demonstrates that slow infusions of tritiated thymidine (3.0 μl/hour) into the brain do not effectively incorporate into organs beyond the brain. This procedure may then be valuable for the selective treatment of patients with brain tumors without jeopardizing the normal proliferative organs.

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14.1 LOCALIZATION OF THE CIRCADIAN PACEMAKER IN THE EYE OF BULLA. G.D. Block and S.F. Wallace*. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

The eye of Bulla gouldiana, like that of Aplysia, expresses a circadian rhythm (CR) in optic nerve compound action potential (CAP) frequency. The Bulla retina contains fewer and larger cells than its Aplysia counterpart and there is better spatial separation between morphologically distinct cell populations. These attributes provide an opportunity for employing long term intracellular recording and selective surgical reduction as techniques for pacemaker localization.

We find that the organized photoreceptor layer is not involved in generating the CAP circadian rhythm. Long term (>24 hr) intracellular recording from photoreceptors fails to reveal membrane potential fluctuations which could account for the simultaneously measured CR in the optic nerve. In most preparations there are small (±2mV) membrane fluctuations, but these appear random and are not statistically correlated with CAP frequency.

Surgical reduction of the retina confirms intracellular results. Paring the retina to its extreme base by removal of the photoreceptor layer does not affect the expression of the CR. Importantly, the period of the CR in the reduced retina (23.9 hr; ±.45) is identical to the CR period of the intact eye (23.8 hr; ±.35), even though only 30-50 cells remain in the fragment. This result supports a similar finding in Aplysia (Strumwasser, 1973) and demonstrates that the CR is generated within individual or small groups of cells and is not due to the mutual interactions of a large population of non-circadian pacemakers as suggested for Aplysia by Jacklet and Geronimo (1971).

Surprisingly, even though the organized photoreceptor layer is removed, retinal fragments remain photosensitive and the pacemaker can be phase shifted by light cycles. Six hr light pulses delivered at Circadian Time 18 cause a 1.1 hr mean phase advance which is similar to the 1.5 hr advance observed in intact eyes. Thus, both a competent circadian pacemaker and its entrainment pathway reside among a small group of cells at the base of the retina. Supported by NS15264 to G. Block.

14.2 ELECTROPHYSIOLOGICAL INVESTIGATION OF CELLS AT THE BASE OF THE BULLA RETINA. S.F. Wallace* and G.D Block (SPON: P. Best) Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

The eye of Bulla gouldiana expresses a circadian rhythm in spontaneous optic nerve impulse frequency (Block & Friesen, 1981). Both the circadian pacemaker(s) and its entrainment pathway reside among a small group of cells at the base of the retina (Block & Wallace, this volume).

We have begun preliminary electrophysiological study of cells at the retinal base. Our first efforts have been directed toward surveying the types of neurons present at the base of the eye with the underlying goal of identifying cells which comprise the circadian pacemaker system.

Intracellular recordings were obtained from isolated Bulla eyes maintained in artificial buffered seawater. To facilitate electrode penetrations, the retina was reduced to its base by removing the lens and then carefully cutting away the surrounding photoreceptor layer. Neurons in the remaining fragment of tissue were impaled with glass microelectrodes (20-40 MΩ) while optic nerve impulse activity was recorded with a suction electrode.

We observed several types of cell responses in the retinal fragment. Most recorded units were spontaneously active (~75%) and responded to illumination with an increased impulse frequency. Spikes from these units were correlated 1-for-1 with compound action potentials in the optic nerve and presumably, were contributing units to the compound optic nerve response. We less frequently observed other cell responses including: 1) a unit which was spontaneously active and hyperpolarized upon illumination; 2) regular beating neurons which did not respond to illumination; and 3) normally quiet cells which responded to illumination with a depolarization accompanied by 2 or 3 spikes which were not correlated with optic nerve impulses.

We also recorded from a non-spiking cell which exhibited a slow 15 mV depolarization in response to illumination. However, unlike intracellular responses from the organized photoreceptor layer, this unit did not rapidly repolarize during sustained illumination. The tonic nature of the reponse makes this cell a candidate for an entraining light-receptor, since the Bulla circadian cell pacemaker can only be phase shifted by long light pulses (>4 hr). Additional intracellular recording using pairwise techniques should reveal the functional relationships between the various cell types at the base of the retina. Supported by NS15264 to G. Block.

14.3 ORGANIZED PHOTORECEPTOR LAYER IS NOT REQUIRED FOR LIGHT RESPONSES IN THREE OPISTHOBRANCH EYES. D.G. McMahon and G.D. Block. Dept. of Biol., University of Virginia, Charlottesville, VA. 22901.

Several opisthobranch eyes have been shown to respond to light with compound action potentials (CAPs) (Aplysia-Jacklet, 1969; Bursatella-Block and Roberts, 1981; Bulla-Block and Friesen, 1981; Navanax-Eskin and Harcombe, 1976). These eyes also express a circadian rhythm in spontaneous CAP frequency. The eyes of another opisthobranch, Haemonea vesicula, exhibit similar behavior. The functional organization of the opisthobranch retina, and specifically the pathways by which light reaches the circadian pacemakers in these retinas is of special interest in the study of biological rhythms (Eskin, 1977; Strumwasser, 1973).

Audesirk (1973) has proposed a model for Aplysia retinal organization in which higher order neurons, driven by photoreceptors are responsible for light-induced CAPs. We were intrigued by evidence in Bulla that higher order cells are, themselves, photosensitive (Block and Wallace, this volume). In order to investigate the role of photoreceptors in the light response we obtained electrophysiological data from three opisthobranch eyes (Aplysia, Bulla, Haemonea).

We find two types of evidence excluding input from the organized photoreceptor layer as critical for the light-induced CAP response. First, simultaneous intracellular recordings from photoreceptors and the optic nerve indicate that the response characteristics of photoreceptors do not match the response in the optic nerve. During a long light pulse (∿30s) receptors first depolarize and then gradually repolarize while CAPs continue at an elevated frequency. Termination of the light pulse has no immediate effect on the receptor membrane potential, but CAPs cease. Similar results were obtained in all three opisthobranchs tested. Second, following surgical reduction of the retina both spontaneous and light-induced CAPS persist (for Aplysia also see Sener, 1972). Histological examination of Aplysia and Bulla retinal fragments reveals that the pigmented layer and all distal segments associated with photoreceptors are absent with only a small number of neurons remaining at the retinal base. Histological analysis of Haemonea retinal fragments is incomplete. These results suggest a reexamination of the present model for opisthobranch retinal organization. Supported by NS15264 to G.D. Block.

14.4 DEMONSTRATION OF IN VIVO AND IN VITRO COUPLED PACEMAKERS IN BULLA GOULDIANA. M.H. Roberts and G.D. Block. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

Bilaterally distributed circadian pacemakers have been identified in a number of organisms. These include the rodent, cockroach, crayfish and several marine gastropods including Aplysia californica (for review see Aschoff, 1981). Many of these organisms have been used to study the properties of pacemaker-pacemaker interactions. However, to date it has not been possible to study a coupled circadian pacemaker system in vitro. We now provide evidence that the two ocular pacemakers of Bulla gouldiana are mutually coupled and that their interactions can be observed in vitro.

Our study of in vitro coupling was prompted by our observations that Bulla, when compared to Aplysia, generally exhibit more coherent and sustained free-running locomotor behavior. Previous work by Lickey et. al. (1980) had shown that poor quality Aplysia free runs were often associated with desynchrony between the two ocular rhythms. We reasoned therefore, that the Bulla eyes, as controlling pacemakers, may always remain in phase, unlike their Aplysia counterparts. In a test of this hypothesis, we determined that even after a month of darkness, the Bulla ocular rhythms were never separated by more than 4 hours. In contrast, results in Aplysia from Lickey's laboratory indicate that ocular phase separations of up to 9 hours appear in as little as two weeks (Hudson, 1978).

This ability of Bulla to maintain ocular pacemaker synchrony in constant darkness can also be demonstrated in vitro. By applying a 12 hour cold pulse to a single eye of an intact Bulla nervous system maintained in vitro, it is possible to induce a 10 hour phase separation between the two ocular rhythms. If the eyes are then allowed to interact through the optic nerves and cerebral ganglion for 36 to 48 hours, the ocular phase separation gradually diminishes to about 4 hours. When a similar cold pulse is applied to a single eye of an intact Aplysia nervous system the 10 hour phase separation remains, even after a number of days of interaction. It appears therefore, that unlike Aplysia, the Bulla ocular pacemakers are tightly coupled and any induced phase difference is quickly reduced.

This is the first demonstration of a coupled circadian pacemaker system in vitro. It should now be possible to investigate directly the dynamics of pacemaker-pacemaker interactions. Supported by NS15264 to G. Block.

14.5 CIRCADIAN PACEMAKER OF BULLA EYE: ELECTRON MICROSCOPY, DYE INJECTION, INTRACELLULAR RECORDING. Jon W. Jacklet and William Colquhoun*. Department of Biology, SUNYA, Albany, NY 12222.

The isolated eye of Bulla gouldiana, the marine bubble snail, contains a circadian pacemaker since the isolated eye expresses a circadian rhythm of compound action potentials (CAP) frequency (Block & Friesen, 1981), similar to the eye of Aplysia. Our electron microscopy of the eye shows it contains about 2500 photoreceptors of 3 types: a large (90 x 30 μm) electron-dense type replete with dense aggregates of 600 Å cytoplasmic vesicles and a large microvillous distal segment; a large clear type with fewer cytoplasmic vesicles and a distinctively different distal segment with larger diameter microvilli; a small slender spindle-shaped receptor with short microvilli and lacking vesicles present in the larger receptors. There are about 70 secondary neurons (20 μm dia), lacking photoreceptor specialization, clustered around the extensive neuropile at the base of the eye. Each secondary neuron has a broad clear axon extending to the optic nerve and neurites branching to the neuropile. Neurons contain rough ER, golgi, dense-core 1000 Å vesicles, and neurotubules similar to secondary neurons of Aplysia eye. Gap junctions between receptors and glial cells, secondary neurons and glial cells, receptor neurites and receptor neurites, and secondary neurites and secondary neurites are common. The optic nerve contains about 2600 axons, perhaps in 1:1 correspondence with the number of receptors and neurons in the eye. Intracellular recording and Lucifer yellow injection show photoreceptor responses of at least 3 types: light induced depolarization without spikes, depolarization with spikes, and depolarization/hyperpolarization with spikes. Dye injections show these responses are from retinal photoreceptors and each has an axon in the optic nerve. Unitary spikes in the photoreceptors are not correlated with the optic nerve CAP but appear in the optic nerve as unitary spikes. The spikes of secondary neurons are correlated 1:1 with the spontaneous and light evoked CAP. Dye injection shows these neurons are clustered at the base of the eye and each has an axon in the optic nerve similar to neurons of the Aplysia eye (Jacklet et al., J. exp. Biol., in press). Secondary neurons of Bulla may be driven to spike by direct current injection, which causes other secondary neurons to fire also and produces an optic nerve CAP. This suggests the secondary neurons are a population of electrically coupled neurons. The neurons are the output neurons of the circadian pacemaker in the eye and very favorable for continued intracellular recording combined with experimental techniques to explore the mechanisms of circadian rhythms. Supported by NSF BNS11154.

14.6 LIMULUS VISUAL THRESHOLD VARIATIONS MEASURED BEHAVIORALLY: DAILY AND SEASONAL EFFECTS. Gerald S. Wasserman. Dept. of Psychological Sciences, Purdue University, West Lafayette, IN 47907.

Limulus ventral eye visual thresholds were measured psychophysically over a period of almost one year. A 12:12 LD schedule was maintained; all testing was done in the afternoon. A seasonal trend was found: In Summer, thresholds clustered around one value. In Winter, thresholds clustered around another value that was 2 log units higher. In Spring and Fall, thresholds were more widely distributed between the two values. This finding suggested but did not prove that two different states exist, that one or the other state prevails in the Summer or Winter, and that both states are present in Spring and Fall. This suggestion was tested by examining response latencies which were also season dependent: In both Summer and Winter, latency followed Pieron's Law and was a declining logarithmic function of intensity with the Summer function shifted toward lower intensities relative to the Winter function. But in Spring and Fall, latencies first declined as intensity was increased, then rose at intermediate intensities, and then fell again at high intensities. This confirms the suggestion that the Spring and Fall results do not come from a single population because a mixture of the Summer and Winter populations should produce a nonmonotonic latency versus intensity function. This mixed function would follow the Summer pattern at low intensities because these would be below the threshold for the Winter type of animal. However as the intensity is raised to the point where Winter type animals do begin to respond, then their average would rise towards the Winter value. Once all animals were recruited, then the function would once again follow a declining pattern. Individual animals exhibited threshold variations that were comparable to the overall group variation. This was true whether one examined data from freshly delivered animals or whether one examined data from animals which had been tested repeatedly in our laboratory for several months. In all seasons, individual animals also exhibited threshold variations from day to day that were as large as the mean difference between Summer and Winter thresholds. A signal detection method was used to examine these day-to-day variations within animals. Large and statistically significant within animal day-to-day variations in signal detectability were found. These data suggest that there are two visual states, that most of the animals are in the more sensitive state in the Summer, that most of the animals are in the less sensitive state in the Winter, and that animals are more evenly distributed between the two states in Spring and Fall. The daily results suggest that any individual can be in either state at any time of the year but that the probability that an animal is in a given state varies with the season.

14.7 REGENERATION OF THE OPTIC TRACTS AND CIRCADIAN PACEMAKER ACTIVITY IN THE COCKROACH. Terry L. Page, Dept. Gen. Biol., Vanderbilt University, Nashville, TN 37235.

It is well established that bilateral severence of the optic tracts (OT) of the cockroach Leucophaea maderae disrupts the circadian locomotor activity rhythm. I found, however, that rhythmicity in DD consistently reappeared in 3-5 weeks (27±5.5 days, N=11) after OT section. After its reappearance the freerunning period (τ) of the rhythm was strongly correlated with τ before surgery (r=0.91), but on the average was slightly longer ($\overline{\Delta\tau}$ = 0.2±0.35h). Also, the phase of the freerunning rhythm (ϕ), projected back to the day of surgery, was correlated with ϕ of the pre-operative rhythm (r=0.77). The average of the absolute values of the differences in phase was 3.4±2.29h (95% conf. limits). It was also found that exposure of animals to light cycles during the first 10 days after OT section (leaving connections between the OL and compound eyes intact) shifted the phase of the subsequent rhythm.

Several observations suggest the return of rhythmicity after OT section is likely due to regeneration of neural connections between the optic lobes (OL) and the midbrain. (1) After removal of the OL, aperiodicity persisted indefinitely (>100 days, N=13). (2) Insertion of a piece of glass coverslip between the OL and the midbrain to slow regeneration prevented return of rhythmicity in 6 animals (>75 days) and delayed it in 3 animals (46±14.9 days). Insertion of the coverslip elsewhere in the head capsule had no effect on the reappearance of the rhythm (N=6). (3) Histological examination of brains following return of rhythmicity showed regeneration of structural connections between the OL and the midbrain. (4) Extracellular recording several weeks after OT section and ocelli ablation showed the return of light evoked activity in axons of the cervical connectives. This activity was abolished if the OT were re-cut. (5) The time course of the return of the light evoked activity paralled the return of behavioral rhythmicity.

These results are consistent with the view that the circadian pacemaker that drives the activity rhythm resides in the OL and controls rhythmicity via neural connections with the midbrain. They also suggest that the oscillation persists in the optic lobe after optic tract section, that it can be entrained by light, and that it is capable of regenerating those connections with the midbrain that are necessary to drive the activity rhythm.

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14.8 NEUROFUNCTIONAL MAPPING OF THE EFFECTS OF ELECTRICAL STIMULATION IN THE SUPRACHIASMATIC NUCLEUS. A.M. Rosenwasser, E. Yadin, N.T. Adler. Dep't of Psychology, University of Pennsylvania, Philadelphia, PA 19104.

The suprachiasmatic nucleus (SCN) of the hypothalamus has been implicated in the control of circadian rhythmicity in a wide variety of neural, endocrine, and behavioral systems. These widespread effects of SCN damage on circadian rhythms are consistent with the neuroanatomical evidence regarding SCN efferents, which appear to be similarly widespread.

Schwartz and coworkers have used the 14C-2-deoxyglucose method of autoradiography to demonstrate the presence in the SCN of a circadian rhythm in neurofunctional activity (Science 205, 723, 1979). Such circadian metabolic rhythms were not seen outside the SCN. On the other hand, electrophysiological studies reveal SCN-dependent rhythms in electrical activity in a number of brain areas. In the present study, we explored the functional connectivity of the SCN by combining 2DG autoradiography with electrical stimulation of the SCN. Chronic stimulating electrodes were aimed unilaterally at the SCN of rats. 30-40 μCi of 14C-2DG were injected ip and stimulation (100-200μA; 0.1 ms pulses at 100 pps) was delivered throughout the 45 min survival period. Animals were sacrificed and their brains were removed and sectioned on a cryostat for autoradiography (10-14 days exposure to x-ray film). Following autoradiography, the sections were stained with cresyl violet or thionin. The results were analyzed using a computer-assisted image processing system (Drexel-Penn system) which allowed the direct super-imposition of radiographic and histological information. Control brains included those in which the electrode placements were found to be outside the SCN as well as similarly treated but unstimulated brains.

Thus far we have analyzed primarily the intrahypothalamic effects of unilateral SCN stimulation by comparing the 2DG uptake of several bilaterally paired nuclei. Increases in 2DG concentration of 10-20% were seen in the anterior hypothalamic nucleus, the paraventricular nucleus, the ventromedial and dorsomedial nuclei, the lateral hypothalamus, and the posterior hypothalamic nucleus on the stimulated side of the brain relative to the unstimulated side. On the other hand, decreased 2DG uptake was noted in the arcuate nucleus on the side of SCN stimulation. These radiographic effects corresponded closely to histologically defined nuclear regions, and were not observed in the control brains. THese results complement and add to the results of SCN connectivity studies using transport of labeled amino acids. It is hoped that by elucidating the functional consequences of SCN stimulation we will help clarify the role of this area in circadian organization.

14.9 ROLE OF THE INTER-CONNECTIONS OF THE SUPRACHIASMATIC NUCLEI IN THE HAMSTER CIRCADIAN SYSTEM. Cheryl L. Sisk and Fred W. Turek. Dept.Neurobiol.& Physiol., Northwestern Univ., Evanston, IL 60201.

The splitting of the circadian rhythm of activity of rodents into 2 separate components provides evidence for multiple oscillators within the mammalian circadian system. In hamsters exhibiting a 2-component split activity rhythm, unilateral lesions of the suprachiasmatic nucleus (SCN) reinstate a single-component activity rhythm. This suggests that an oscillator or population of oscillators within a single SCN can control a single activity component. Neurons within each SCN project to the contralateral nucleus and their axons cross the midline within the underlying optic chiasm. The function of these SCN inter-connections is unknown. To determine if these fibers provide neural coupling between putative circadian oscillators, midsagittal knife cuts which severed the SCN reciprocal connections were made in male golden hamsters which had developed a split activity rhythm during prolonged exposure to constant light. Preoperatively, each of the split activity components consisted of a dense band of activity usually 1-2 hr in length. Following the knife cut, it was frequently observed that the 2 activity components became more diffuse and of longer duration, consisting of many small activity bouts per component with a total activity duration of up to 5 hr per component. In other hamsters, the postoperative phase relationship between the 2 components was noticeably different from the characteristic preoperative 180° antiphase. Other consequences of the knife cut which were observed included changes in phase of the 2 components with respect to time of day, and either an increase or a decrease in period of the components. Importantly, postoperatively the 2 components were never seen to free run with differing periods and were always stably in phase with each other. This range of responses was observed in hamsters in which the knife cut was on the midline and had resulted in minimal or no damage to either of the SCN. Complete abolition of the split activity pattern occurred only in hamsters found to have sustained substantial unilateral SCN damage. These results suggest that (1) the neural connections between the SCN participate in the integration of individual activity bouts into unified activity periods which are under circadian control, (2) the connections may be involved in regulating certain parameters of coupled circadian oscillators such as period and phase, and 3) in the absence of the SCN-SCN neural connections which traverse the optic chiasm, other neural or hormonal pathways exist which allow for the stable coupling of the 2 activity components in the split circadian system.

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14.10 PERINATAL ENTRAINMENT OF HAMSTER CIRCADIAN RHYTHMS. Fred C. Davis* and Roger A. Gorski. Laboratory of Neuroendocrinology of the Brain Research Institute & Department of Anatomy, UCLA School of Medicine, Los Angeles, California 90024.

Study of the development of the neural mechanisms which underly circadian rhythm generation should contribute to a more general understanding of this important biological problem. A useful approach to this question is to determine when during development circadian mechanisms first become functional. The present study demonstrates that the activity/rest rhythm of hamster pups is entrained by maternal rhythmicity at some age prior to weaning (postnatal day 18), and suggests that this entrainment actually begins before birth. Wheel-running activity was recorded from hamster pups following weaning at 18 days of age. Continuous recording of activity for several weeks in constant dim light (dim LL, < 1 lux) provides a precise determination of the phase of activity onset on the day of separation from the mother. Pregnant hamsters were maintained in dim LL throughout gestation and lactation, with some hamsters receiving 10% heavy water (D_2O) for drinking water. The D_2O was used to lengthen freerunning period in some of the mothers. At weaning four pups from each of four litters (2 H_2O and 2 D_2O) were individually isolated in wheel-running cages. Phases of activity onset of the 16 pups occured at widely scattered "clock times" (probability of random distribution >0.1, Rayleigh test). The scatter, however, is related to differences in the mothers' phases of activity onset resulting from differences in their freerunning periods. Relative to "mother time" pups were found to be synchronized ($P<.001$), phase leading their mothers by an average of 1.6 hr. This relationship between mother and pup onsets could have been established prenatally, postnatally or during both times. To test if maternal entrainment of pup rhythms occurs before birth, mothers were maintained on one of two 14/10 light/dark cycles with lights off 180° out of phase. Pups from these mothers were raised in dim LL by foster mothers from only one of the prenatal LD cycles. If the pups possessed entrained rhythmicity at birth, the groups would be either "in phase" or "out of phase" with their foster mothers. At weaning, "in phase" pups (n=12) were synchronized ($P<.001$) either with respect to "clock time" or "foster mother time", phase leading the mothers by an average of 1.7 hr. "Out of phase" pups were not synchronized ($P>.1$) either with respect to clock or foster mother time. The synchronization among "in phase" pups suggests an entraining influence by the foster mothers which did not occur with the "out of phase" pups. This difference can only be attributed to the difference in prenatal phase, suggesting that entrainment of rhythmicity in the pups occurs before birth. Supported by NRSA HD05916 to FCD and NIH grant HD01182 to RAG.

14.11 CIRCADIAN RHYTHMS OF BODY TEMPERATURE AND WHEEL RUNNING IN THE TURKISH HAMSTER(Mesocricetus brandti). H. E. Albers, D. S. Carter*, J. M. Darrow* and B. D. Goldman*. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Much of the empirical base for current views of the functional and physiological organization of the mammalian circadian timing system has been obtained from studies using the Syrian (golden) hamster (Mesocricetus auratus). In view of the lack of data in other hamster species we have begun to examine the circadian system in the closely related Turkish hamster (Mesocricetus brandti). In each of 8 male Turkish hamsters entrained to LD 16:8, core body temperature was telemetrically recorded at hrly intervals for 27 consecutive hr and wheel running was continuously monitored on an event recorder. Body temperature varied between 36.3 ± 0.1 and 38.4 ± 0.1°C over the 24 hr day. The initial rise in temperature tended to anticipate the 8 hr dark phase while activity began shortly after dark onset. The acrophase of the activity rhythm occurred ~2.5 hr after dark onset, while temperature maxima were consistently found 1.74 ± 0.12 hr later into the dark phase, as determined by a least squares sine wave fitting technique. To determine the characteristics of the free-running circadian activity rhythm records were obtained from 14 male and female hamsters initially maintained in LD 16:8 and subsequently exposed to constant light (LL; 40 lux) for 2 months. Several hamsters displayed precise free-running circadian patterns of activity similiar to those reported for the Syrian hamster; however, the activity rhythms of the remaining hamsters were less regular displaying spontaneous changes in period and/or phase. Time series analyses indicated that the activity pattern of each hamster had a statistically reliable period within the circadian range (24.38 ± 0.14 hr). Following 30 days of re-entrainment to LD 16:8, all hamsters were blinded and castrated to determine if the spontaneous changes in the free-running patterns were produced by either LL exposure or endogenous changes in gonadal steroids. Activity records for the 2 months following surgery suggested that free-running rhythms were more disrupted than during the previous LL exposure. Time series analyses revealed 2 different statistically reliable circadian periods within the activity records of each of 8 hamsters. In summary, Turkish hamsters maintain precise circadian patterns of both body temperature and activity when entrained to an LD cycle. However, under constant conditions the activity rhythm exhibits spontaneous perturbations which are not the simple result of the disruptive effects of LL or changing levels of gonadal steroids. These data emphasize the importance of comparative studies of circadian organization and suggest that functionally important differences may exist in the circadian system of even closely related species.

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14.12 SPLIT FREE-RUNNING ACTIVITY RHYTHMS IN THE GOLDEN HAMSTER: DIFFERENTIAL RESPONSES OF THE TWO COMPONENTS TO SINGLE 6-HOUR DARK PULSES. Jodi G. Lees* and John D. Hallonquist. Dept. of Zoology, University of Toronto, and Dept. of Psychiatry, Mount Sinai Hospital, Toronto, Ontario, Canada.

The phenomenon of splitting of the locomotor activity rhythm in the golden hamster has been well documented. Splitting is generally considered evidence for the presence of two mutually coupled oscillators underlying this rhythm. Conditions that result in splitting are well known (eg., environmental lighting, hormonal milieu of the subject), however, little is known regarding the formal properties of the two components while in the split condition. Phase shift experiments may reveal certain characteristics of the underlying pacemaker system(s).

Eleven male golden hamsters were maintained in constant light (LL) of 100-175 lux to encourage splitting. Six of these subjects displayed split wheel-running activity rhythms after 60 to 122 days in LL. Five subjects (2 split, 3 non-split) were first exposed at various circadian times to a single 2-hr. pulse. The effects of this pulse were minimal, therefore all 11 subjects were subsequently exposed to a single 6-hr. dark pulse at various circadian times after 128 to 137 days in LL. A second 6-hr. dark pulse was presented 6 wk. after the first 6-hr. pulse, allowing sufficient time for the rhythms to stabilize.

The 6-hr. dark pulses had profound effects on the split rhythms. These effects appeared to depend on the time of pulse presentation relative to activity onset, as well as an interaction between the two components. Of greatest interest were the differential responses of the 2 components of split rhythms. Components often shifted in opposite directions. Components shifting in the same direction did so by different amounts. In some cases, immediate and steady-state phase shifts of a single component occurred in opposite directions. Occasionally these shifts were as large as 7 hr. In most cases transients resulted in the reestablishment of the pre-pulse phase relationship. In a few instances a single split component was not expressed for 1-17 days post-pulse. Another effect was the merging of 2 split components as a result of unequal phase shifts followed by changes in periodicity from $<$ 24 hr. to $>$ 24 hr. Effects of 6-hr. dark pulses on non-split rhythms included phase shifts, temporary splitting lasting up to 38 days post-pulse, or no effect at all. Transients were smaller in size and persisted for fewer days in non-split rhythms.

Compared to well-integrated non-split rhythms, split rhythms appear to be more labile in their response to dark pulses. The differential response of the split components provides further evidence for multioscillator control of the circadian locomotor activity rhythm in the golden hamster. (NSERC grant to N. Mrosovsky, Dept. of Zoology, U of T, & NSERC graduate award to JGL.)

15.1 RECOVERY TIME AND TACTILE LEARNING FOLLOWING SM-1, SM-2 AND SM 1+2 LESIONS IN THE RAT. S. Finger, T. Hart* and E. Jones*. Psychol. Dept., Washington Univ., St. Louis, MO 63130.

In an earlier study from this laboratory (Finger & Reyes, Physiol. Beh., 15:289, 1975) rats with simultaneous bilateral lesions of Sml+2 were tested for acquisition of a series of 5 tactile discriminations. The animals with ablations performed worse than sham operated rats (and comparably to each other) whether given weeks, months or 1-2 years for recovery.

The question asked in the present study was whether persistent deficits would also be witnessed after lesions restricted to just Sm-1 or just Sm-2, or whether there would be observable recovery over time after these "subtotal" sensorimotor cortex lesions. To answer this question, 52 rats with bilateral lesions of Sm-1, and 47 with bilateral lesions of Sm-2, were tested on the same battery of tactile discriminations that was used previously. The surgeries were performed when the animals were 3-4 mo. of age. Some rats were given 1 or 2 weeks for recovery; some were allowed 1 or 6 mos. for recovery; and some remained in their home cages for 1 or 2 yrs. before starting testing.

The rats with Sm-1 lesions performed poorly when the recovery period was 1 yr. or less, but scored within the control group range in the 2-yr. recovery condition. Sm-2 animals also did poorly with the shorter recovery periods, but their learning scores appeared "normal" in both the 1 and 2 yr. recovery conditions.

These data suggest that the capacity for rapid tactile learning after Sm-1 lesions is dependent upon Sm-2 remaining intact and vice-versa. Whether the animals adapted new strategies (e.g., rubbing a stimulus a bit harder), or whether they were exhibiting "true recovery," however, could not be discerned under the conditions of this experiment. Nor is it clear at the present time why such extensive recovery periods were required before the postoperative learning scores of the Sm-1 and Sm-2 rats descended into the control group range.

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15.2 DEFINITION OF SOMATOSENSORY TRANSITIONAL ZONES (TR) IN THE SENSORIMOTOR (SI-MI) CEREBRAL CORTEX OF RATS. Wally Welker, Kenneth J. Sanderson, and Georgia M. Shambes. Department of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Details of afferent and efferent projections to and from somatosensory cortex in rats have not yet been defined precisely. We examined light touch somatosensory afferent and movement-elicited efferent projections of SI, MI and the transitional (TR) zones between them. Subjects were albino rats anesthetized with ketamine hydrochloride. Tungsten ball-tip microelectrodes (impedances of 0.3-1.0 MΩ) (a) recorded single- or multiple-unit responses in SI,TR & MI evoked by punctate threshold mechanoreceptive stimulation of body parts and (b) electrically stimulated laminae V & VI to evoke body movements (15-25 msec biphasic pulse trains, each pulse=100 μsec; via optical stimulus isolators at 2 trains/sec; thresholds=4-250 μA). In-depth microelectrode micromapping techniques at high puncture densities yielded micromaps of receptive fields (RF's) and movement fields (MF's). Recording and stimulating sites (marked by microlesions) were verified microscopically on frozen thionin-stained sections. RESULTS: The TR zones lie between the cell dense patches in SI that receive projections from head & forelimb and from forelimb & hindlimb, as well as between granular SI cortex and agranular MI cortex. The RF's in any portion of TR are similar in general body location to those in adjacent SI. Compared to RF's in MI & SI, TR RF's have intermediate: (1) force-amplitude-velocity thresholds, (2) size, (3) sharpness of their boundaries, (4) degree of somatotopy, and (5) cortical depth of best activation of multiple units. TR latencies to mechanical stimulation overlap those to SI & MI (for hand: SI=14-18 msec; TR=13-18 msec; MI=13-20 msec). MI "motor" maps are similar to those of Hall & Lindholm (Brain Res. 66:23-38,1974) and Donoghue & Wise (Neuroscience Abstracts, 5: 18,1981), exhibiting detailed musculotopy. Afferent inputs to MI and the rostral forelimb motor area (Neafsey & Sievert, Brain Res.232:151-156,1982) derive from RF's that are similar to the movement fields elicited from these same cortical regions. SI projections were similar to those of C. Welker (Brain Res. 26:259-275,1971). But, considerable individual variations occurred in somatotopic projections to SI and musculotopic projections from MI. SI afferent projections (at mean depths of 630μ) partially overlap MI efferent projections (at mean depths of 1320μ) in hindlimb and forelimb regions, confirming Hall & Lindholm (1974) and Donoghue & Wise (1981). These studies contribute further information to the many ongoing studies of finer projectional details of connections and functions of sensorimotor systems.

15.3 SIGNIFICANCE OF TOPOGRAPHY AND SUBMODALITY IN THE ORGANIZATION OF BRODMANN'S AREA 1 IN SmI OF MACACA. Mary Carlson, Shao Dianhua* and Xu Bingxuan*. Institutes of Physiology and Brain Research, Academia Sinica, Shanghai, PRC and Dept. of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

The representation of the cutaneous input from the hand in the postcentral gyrus in Macaca has been variously described. Paul et al.('71) proposed separate hand projections aligned serially in areas 3 and 1, and with the digit tips pointing rostral; Kaas et al.('79, '80) also proposed separate hand zones for 3 and 1, but organized as mirror images with digit tips at the 3a-3b and 1-2 borders('71); Whitsel et al.('71) proposed a single cutaneous core zone within 3 and 1. The relative position of the digit receptive fields(RFs) in area 1 remains the most disputed issue in the topographic organization of the hand area/areas.

In an attempt to resolve this issue we recorded in several species of Macaca, using anesthetized and unanesthetized preparations and multi- and single neuron techniques. We found that the glabrous surface of the digits projected to area 3 and the hairy digit RFs projected to area 1 but not to area 3. RFs on the digit tips projected to the 3a-3 border, the area 1-2 border and also to the 3-1 border. However, RFs from both proximal and distal digits were frequently interspersed throughout area 1 in the anteroposterior plane. RFs on distal hairy digits projected to the area 3-1 border and proximal digit RFs to the area 1-2 border. Although RFs of glabrous digits were frequently adjacent to those on the dorsal, hairy surface of the same distoproximal location in area 1, the topography of hairy RFs was more evident than for glabrous RFs. In studies of awake Macaca we found neurons in area 1 responded to either glabrous or hairy digit skin or to joint manipulations, in close proximity in single perpendicular penetrations. The organization of submodalities in area 1, as in area 2, appears more important than topography.

Our studies of Macaca SmI are in agreement with some components of all previous studies but differ in emphasizing the heterogeneity of the representation in area 1. We conclude that 1) cytoarchitectural distinctions between areas 3 and 1 in primates are associated with different projections of glabrous and hairy skin and 2) glabrous RFs in area 1 of primates represent an ectopic projection within the original hairy RFs, both in Macaca and the prosimian, Nycticebus (Carlson and FitzPatrick, '82)

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15.4 CALLOSAL CONNECTIONS OF POSTCENTRAL SOMATOSENSORY CORTEX IN OWL AND MACAQUE MONKEYS: I. THE RELATION OF TERMINATIONS AND PROJECTING CELLS TO THE SEPARATE BODY MAPS IN AREAS 3b, 1 AND 2. H.J. Gould, III, C.G. Cusick*, T.P. Pons, H.P. Killackey, and J.H. Kaas. Dept. of Psych., Vanderbilt Univ., Nashville, TN 37240, Dept. of Psychobiol., Univ. of Calif., Irvine, CA 92717, and Dept. of Anat., LSU Med. Ctr., New Orleans, LA 70112.

Recently, it has become apparent that each of the four architectonic fields of the traditional "First Somatosensory Area" of monkeys corresponds to a separate representation of the body (e.g. Kaas et al., [Science, 204: 521, 1979]). The goal of the present study was to see how callosal connections relate to these separate representations. Axonal terminations and cells of projection were revealed by horseradish peroxidase (HRP) histochemistry following multiple injections (.04 to .2 μl typically at 0.5 to 1.5 mm spacing) of the tracer into postcentral parietal cortex of one hemisphere and extensive microelectrode mapping (350 to 575 recording sites) of the body representations in Areas 3b, 1, and 2 of the other hemisphere. Reference microlesions were placed to facilitate correlation of recording sites with body cytoarchitecture and locations of labeled neurons and axon terminations. Brains were cut in the parasagittal plane or flattened and cut parallel to the surface. The flattened brains allowed the two dimensional surface view of the connection pattern to be directly appreciated.

The results lead to several major conclusions. 1) The connection patterns for New and Old World monkeys were roughly similar. 2) The separate representations differed markedly in the overall magnitude of callosal connections. Callosal connections were sparse for Area 3b, moderate for Area 1, and very dense for Area 2. 3) In all three Areas, projecting and receiving areas were correlated, although projecting regions tended to be somewhat more broadly distributed. 4) The connections were unevenly distributed across body parts in the three representations. For Area 3b, the most dense connections were largely restricted to the representations of the trunk and parts of the face. However, scattered labeled cells were found elsewhere even occasionally in the representation of the glabrous hand. In Area 1, the pattern was similar, but the limb regions generally contained more label than in Area 3b. In Area 2, more densely labeled regions included all major body parts including the hand and foot. Since the representations in Areas 3b, 1, and 2 are serially connected, the results suggest that the callosal transfer of information is largely delayed until it reaches the Area 2 representation, where transfer occurs for all body parts. More limited transfer concentrated on some body parts occurs for the representations in Areas 3b and 1. Supported by NIH Grant NS16446.

15.5 CALLOSAL CONNECTIONS OF POSTCENTRAL SOMATOSENSORY CORTEX IN OWL AND MACAQUE MONKEYS: II. LAMINAR AND AREAL DISTRIBUTION PATTERNS. C.G. Cusick*, H.J. Gould, III, T.P. Pons, H.P. Killackey, and J.H. Kaas. Dept. of Psych., Vanderbilt Univ., Nashville, TN 37240, Dept. of Psychobiol., Univ. of Calif., Irvine, CA 92717, and Dept. of Anat., LSU Med. Ctr., New Orleans, LA 70112.

In the previous abstract callosal connections were related to the separate body representations in Areas 3b, 1 and 2 (Gould et al.). Two further aspects of the organization of callosal projections are laminar patterns of origin and termination, and the tangential clustering of connected regions within architectonic areas. These features of the somatic callosal connections were studied in the material described previously. The projecting cells had similar laminar locations in each of Areas 3b, 1 and 2. Most labeled neurons were found in the lower part of layer III, fewer were found in upper layer III, and an occasional one in layer V. The great majority of callosally projecting neurons were pyramidal cells. The basal dendrites of well-defined cells showed a variety of morphologies. In some cases, basal dendrites formed a radiating field around the soma, in others, basal dendrites extended horizontally in the lamina of the cell body, and still other neurons had basal dendrites descending below the level of the cell body. There was no clear preponderence of one type of dendritic morphology. The distribution of anterograde HRP label was also similar in the 3 architectonic fields. Anterograde label was densest in layer IV, decreased in density in the lower part of layer III and extended to layer I. The anterograde label appeared strongest in a region traditionally considered to be part of Area 2. In this heavily labeled region, the terminal field in layer IV was very conspicuous, and it was clear that the dense terminal layer was spatially separate from the layers containing projecting cells.

The areal pattern of distribution was reconstructed from parasagittal sections and was directly apparent in the flattened sections. In the trunk region, 3-4 elongated patches were seen, one of which appeared to be roughly associated with the 3b/1 border as defined physiologically and anatomically. Patches of input were also seen in the face region of areas 3b and 1, but evidence for a regular pattern was lacking. These findings suggest that: 1) although the origin and termination zones roughly correspond in their tangential distribution, the laminar patterns are not precisely homotypic. The presence of a dense terminal field in layer IV suggests that stellate cells found in this layer form a synaptic link in the interhemispheric transfer of information. 2) The bands or patches of callosal neurons and terminations do not form simple repeating shapes but may be complexly organized in relation to peripheral receptive fields and cytoarchitectonic areas. Supported by NIH Grant NS16446.

15.6 STIMULUS-EVOKED METABOLIC ACTIVITY IN THE VENTROBASAL THALAMUS OF MONKEYS. S.L. Juliano, B.L. Whitsel & P.J. Hand. Dept. Physiol. & Dental Res., Univ. of N. Carolina, Chapel Hill, NC and Dept. Animal Bio., Sch. Vet. Med., Univ. of PA, Phila., PA.

Although much information exists regarding the body representation in the somatosensory thalamus, neurophysiological investigations of this region have not provided maps possessing the fine grain of those available for the somatosensory cortex. The (^{14}C)2-deoxyglucose (2DG) technique provides an excellent means, in the same animal, for studying the detailed relationship between the peripheral mechanoreceptor sheet and its representation in both the ventrobasal complex (VB) of the thalamus and in the cortical fields which receive VB projections. The experiments in this study mapped the 2DG activity evoked in VB by precisely controlled mechanical stimuli applied to different regions of the body. Five Cynomolgous monkeys were used; details of the method have been reported previously (Juliano, et al., J. Neurophys., 46, 1981). Different modes of somatic stimuli were applied unilaterally to varying proximo-distal regions of the upper and lower limbs. These stimuli were servo-controlled: flutter vibration (15 hz), brush strokes (27 cm/sec) and joint rotation (12^{o}/sec). The VB labeling of all animals exhibited certain common attributes. 1) The labeling was dorsoventrally extensive, and occupied a crescent-shaped territory. 2) Within the crescent of label, fluctuations in optical density exist, revealing regions of dense metabolic activity embedded in larger territories of more diffuse labeling. 3) The stimulus related labeling occupies long antero-posterior distances (1-1.5 mm). The distribution of 2DG labeling shifted systematically within the medio-lateral dimension of VB as the locus of stimulation changed from one body region to another. The topographic relationships indicate that the lower extremity is represented most laterally, the face most medially, and the hand in a central position surrounded both medially and laterally by the arm. For 3 animals, the 2DG patterns were compared to the distribution of HRP labeled cells in VB obtained from injections into functionally characterized fields within SI. This comparison revealed that injections of HRP into an SI region representing the body part stimulated in a 2DG experiment retrogradely labeled cells in a territory of VB closely matching that labeled in the 2DG experiment. The significance of the arrangement of the 2DG label into intermittent dense cores embedded within crescents of less dense label is not immediately apparent. The possibility exists that the dense core labeling corresponds to aggregates of VB neurons whose receptive fields (RFs) were maximally engaged by the stimulus employed, while the surrounding less dense field of activation corresponds to neuron aggregates whose RFs are less completely engaged by the same stimulus. (Supported by NS 10865 and DE 02668)

15.7 ARE THERE INTERNEURONS IN THE THALAMIC SOMATOSENSORY PROJECTION NUCLEUS IN THE RAT? J. Wells, T. J. Mathews* and M. A. Ariano. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Evidence has been accruing that the thalamic projection nucleus for the somatosensory system in the rat (ventral posterolateral nucleus - VPL) contains no interneurons. Confirmation of the absence of interneurons is important for at least two reasons. Since other projection nuclei have interneurons, it would indicate that the rat VPL must have developed unusual mechanisms for the maintenance of somatotopy and modality specificity. Secondly, the lack of interneurons may play a role in the reactive synaptogenesis that occurs in VPL following lesions to its afferent innervation. We have attempted to show the presence of interneurons in the rat VPL by injecting the cortex with HRP in order to determine if there is a population of unlabeled neurons in VPL. In addition, since there is a high concentration of GABA receptors in VPL which might indicate GABA-containing interneurons, we have stained VPL with an antibody to glutamic acid decarboxylase (anti-GAD) to determine if there is a special population of GAD-containing neurons within VPL.

Following injections of HRP into SI cortex, Saporta and Kruger (J. comp. Neur. 174:187, 1977) found all neurons appeared labeled within a coherent band of VPL and suggested there were no neurons intrinsic to VPL. We have modified their experimental paradigm by using thinner sections so that unlabeled neurons would not be obscured by heavily labeled neurons or by the antegrade transport of HRP from cortical neurons, and by using large injections into both SI and SII cortex. When we used 10-15 µm thick sections after large HRP injections of cortex stained by the tetramethyl benzidine method, all of the neurons appeared to contain reaction product. A few neurons remained equivocal, however, in 1-2 µm sections randomly selected throughout VPL and stained by the glucose oxidase-diaminobenzidine method there again appeared to be no unlabeled neurons within the labeled zone of VPL. Therefore, if unlabeled neurons do exist, they must form a very small population.

After staining with anti-GAD there were many neurons which appeared darkly labeled. These neurons were randomly scattered throughout VPL and were the size and shape of typical VPL projection neurons. There were too many neurons stained by the anti-GAD reaction to account for the unlabeled or equivocal population observed after HRP injections. Thus, there may be a subpopulation of VPL neurons that perhaps use GAD for the synthesis of transmitters such as glutamate. These studies further support the evidence that the VPL in the rat does not contain interneurons. Supported in part by PHS 5429-17-3 to J.W and BNS 81-02648 to MAA.

15.8 PATTERNS OF CORTICAL PROJECTION UPON THE VENTROBASAL THALAMIC COMPLEX OF CAT, E. Ramon-Moliner, Department of Anatomy and Cellular Biology, Shool of Medicine, University of Sherbrooke, Sherbrooke, Québec, Canada, J1H 5N4.

Following injections of labelled aminoacids in various points of the frontal lobe of cat, the following patterns were found: (1) The pericruciate cortex as a whole sends fibers to VA and rostral VL, to ventral LP, to medial VPM, to VPLm (but not to VPLl, unless rostrolateral sigmoid cortex is included), to CL (profusely), to CM (sparsely), to the perifascicular region and to certain unnamed islands within the pretectal region (see below). (2) Following multiple microinjections in pericruciate (anterior, lateral and posterior sigmoid) gyrus, the lack of projections to caudal VL and lateral VPM results in a dark, negatively outlined area, surrounded by a "white halo" of labelled fields, when using dark field illumination. Its shape is ovoid and lies within the VB complex. It could be partially due to failure to inject the deeper intracruciate cortex. It is present, though less extensively, following single injections in intracruciate, coronal and anterior ectosylvian cortices. (3) The lateral sigmoid cortex projects to caudal VL, VPL, lateral VPM, caudal CL and medial n. ruber. The lateral sigmoid projection to caudal VL also outlines a nonlabelled oval field within which certain circumscribed spherical labelled fields ("ventrobasal islands") can be seen. (4) An injection confined to the cortex within the depth of the cruciate sulcus resulted in labelling of ventral LP, caudal VL, CL, CM, and the intermediate perifascicular region. A negatively outlined area, devoid of afferents, also resulted from this intracruciate injection. (5) The rostral interhemispherical cortex, including gyrus proreus and genualis, projects selectively to MD, centralis medialis, dorsomedial LD and paratenialis. (6) All the injected areas in the rostral forebrain send fibers to the perifascicular region and to some unnamed scattered small fields in the pretectal region. This projection takes place by means of collaterals of the cerebral peduncle which form the bundle of Ramón y Cajal (Trab. Lab. Invest. Biol. 25: 129-143, 1928) or prerubral bundle (Brain Res. 170:1, 1979; and 194: 484, 1980). The proreus-genualis, medial precruciate, and lateral pericruciate (sigmoid) cortices project respectively to dorsal, intermediate, and ventral perifascicular regions. (Supported by grant MA6820 from the National Research Council of Canada).

15.9 GAD IMMUNOREACTIVE NEURONS IN THE VENTRAL POSTERIOR NUCLEUS OF CAT AND GALAGO. G.R. Penny, D. Fitzpatrick*, D. Schmechel* and I.T. Diamond. Depts. of Psychology and Neurology, and Neurobiology Program, Duke University, Durham, NC 27706.

This report is a companion to our abstract on the lateral geniculate nucleus in this volume. In this study we use the presence of glutamic acid decarboxylase (GAD) immunoreactivity to identify neurons in the ventral posterior nucleus which presumably use the inhibitory neurotransmitter GABA. GAD immunoreactivity was demonstrated by PAP and avidin-biotin immuno-peroxidase methods. We find that these neurons form a distinct class of thalamic cells on the basis of their size and morphology. In both the cat and the prosimian Galago GAD positive cell bodies are found distributed throughout the nucleus. Counts of the relative number of GAD positive cells and unlabeled Nissl stained neurons indicate that the GAD containing neurons make up about 25-30% of the neurons in the ventral posterior nucleus of both species. In addition, the ventral posterior nucleus is characterized by a dense population of GAD terminals and is divided by GAD-free fiber lamellae. The GAD positive neurons are significantly smaller than the unlabeled, Nissl stained neurons in the same regions. In the cat, a sample of GAD cells has a mean soma area of 150 um^2 and unlabeled Nissl stained neurons in the same area have a mean size of 308 um^2. In the Galago the mean size of a sample of GAD cells is 90 um^2 and unlabeled Nissl stained neurons in the same region have a mean size of 178 um^2. Although most of the unlabeled, Nissl stained neurons are larger than the GAD positive neurons, it is of interest to note that there are some non-GAD neurons that are small and are in the size range of the GAD positive neurons. We have previously reported that neurons of this size-class may project to the superficial cortical layers. The GAD positive neurons display few primary dendrites (2-4), and these dendrites tend to be oriented parallel to the fiber lamellae which transect the ventral posterior nucleus. The similarities in size and proportion of GAD positive neurons in the ventral posterior nucleus and the lateral geniculate nucleus of these species, supports the idea that GABAergic inhibitory neurons are a consistent feature of the organization of sensory relay nuclei in the mammalian thalamus. Whether GAD positive neurons are, in fact, local circuit neurons is currently being tested by combining retrograde labeling with immunocytochemistry.

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15.10 SOMATOTOPIC ORGANIZATION OF THE VENTROPOSTERIOR NUCLEUS OF THE SQUIRREL MONKEY (Saimiri sciureus). R. J. Nelson, J. H. Kaas, M. M. Merzenich, R. W. Dykes and M. Sur*. Depts. of Psychology and Anatomy, Vanderbilt Univ., Nashville TN 37240.

To determine the detailed somatotopic organization of the ventroposterior nucleus of the thalamus in squirrel monkeys, we used multi-unit recording techniques to record in each of the three cardinal planes because reconstructions made from penetrations in any one plane yield incomplete topographic sequences of the body surface representation.

Multi-unit responses to natural stimulation were characterized for recording sites at 50 um intervals under ketamine anesthesia. Receptive fields were mapped for sites responding to tactile stimulation. Subsequent histological reconstruction of electrode tracks in each case confirmed that sites responding to cutaneous stimulation were confined to thalamic nuclei VPL and VPM. Cells at recording sites dorsal and ventral to VPL were activated by non-cutaneous input.

Results show that there is a single systematic representation of the contralateral body surface in VP although there is also a submodal segregation in this region of the thalamus (Dykes et al., Neuroscience 6(8):1687-1692, 1981). The dorso-caudal portion of VPL receives input from the axial body where body surface locations from cranial to caudal on the trunk are represented from medial to lateral. Ventral to the trunk representation, the proximal extremities and the dorsal surface of the hand and foot are represented. Most of the ventral and rostral portions of VPL are occupied by the representation of the glabrous hand and foot. Each digit of the hand and foot is represented in a smaller region which, when reconstructed, appears disc-shaped, elongated in the antero-posterior dimension and internally concave, conforming to the external curvature of VPL. The volar pads are represented dorsal to the representation of the digits for both hand and foot. VPM receives input from the face with the lips represented ventrally and rostrally while the remainder of the face and head represented dorsally and caudally adjacent to the trunk representation in VPL.

Reconstructions of VP in each plane show that no single cardinal plane contains a strictly continuous representation of the body surface; there are discontinuities in the representation in each cardinal plane, which result in abrupt shifts in the receptive field sequence for sites recorded as little as 100 um apart. These abrupt shifts are most likey to occur in the medio-lateral plane. When discontinuities do occur in the thalamic representation of the body surface they correspond with naturally occurring body surface discontinuities such as those between the digits of the hand and foot.

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15.11 CORTICO-LIMBIC PATHWAY FOR TOUCH: CONNECTIONS VIA SOMATOSENSORY CORTICAL FIELDS IN THE LATERAL SULCUS OF THE MONKEY, D. P. Friedman, E. A. Murray and M. Mishkin. Lab. of Neuropsychology, NIMH, Bethesda, MD 20205

Combined damage to the amygdala and hippocampus results in a severe tactual memory deficit in monkeys (Murray, E. A. and M. Mishkin, Neurosci. Abstr., 7: 237, 1981). Yet the pathways by which somatosensory information could reach these limbic structures and thereby account for their participation in tactile memory processes have not been established. In a search for such pathways, single- and multi-unit recording techniques were used to identify the specific cortical fields in the parietal lobe and the lateral sulcus of macaques that are activated by somatic input. These fields include the first and second somatic sensory areas (SI and SII), areas 5 and 7b, the retroinsular area (Ri) and the granular and dysgranular insular fields (Ig and Id). After a particular field was mapped, an injection of either tritiated amino acids or horseradish peroxidase was made into the hand or digital representation within it to trace its connections.

The anterograde and retrograde axonal transport experiments together demonstrate reciprocal connections between SII and Ri, SII and area 7b, SII and Ig and Id, and Ri and Ig. We have confirmed the previously reported reciprocal connections of SI with SII, and demonstrated reciprocal projections between area 5 and both Ri and area 7b.

The laminar pattern of termination of these projections suggests the sequential order in which the somatic sensory fields process information. In the visual system, "forward" cortico-cortical projections (i.e., projections directed away from striate cortex) are characterized by a heavy input to layer IV, while "backward" projections completely avoid layer IV and instead have a characteristically heavy input to layer I. By analogy with the visual system, an analysis of the laminar pattern of termination in our material indicates that the forward sequence of projections is: SI to SII and area 5, area 5 to Ri and 7b, 7b and Ri to SII, and SII and Ri to Ig; SII also projects to Id. Finally, we have confirmed previously reported projections from Ig and Id directly to the amygdala and indirectly to the hippocampus via the rhinal cortex.

These data demonstrate, for the first time, a series of parallel tactile processing pathways that converge on the insular cortex, via which somatosensory information can then reach the limbic structures of the temporal lobe. The entire set of connections could serve as a hierarchically organized cortico-limbic pathway for tactile perception and memory.

15.12 CENTRAL REPRESENTATION OF A SPECIALIZED MECHANORECEPTOR ARRAY IN THE WING OF THE BAT. J.M. Zook and B. C. Fowler*, Coleman Lab. University of California, San Francisco, Ca. 94143.

The bat's wing is an elaborated hand in which the elongated digits and arm support six web membranes. For more than a century prior to the discovery of echolocation it was commonly believed that a special tactile sensitivity of the wings enabled bats to fly and navigate in the dark.

We have begun to investigate the nature and distribution of wing cutaneous receptors and their representation in the central nervous system by recording within somatosensory cortex and by histological examination of the wing. Primary somatosensory cortex was mapped with closely spaced microelectrode penetrations in anaesthetized bats of the species Antrozous pallidus.

These experiments reveal a number of unique features both of the cortical representation of the wing and of the mechanoreceptor population within the wing. More than a fifth of the body surface representation in somatosensory cortex is devoted to a representation of the wing. Although the membranous web is very thin, both dorsal and ventral wing surfaces are completely represented. The alternating order of digits and web membranes across the wing surface is not maintained in the cortical representation, i.e. digits are represented in one area and webs in another. However, within each of these areas, the topographic order of the relevant surface is maintained. Most units within the area of web representation are characterized by slowly adapting response. Optimal stimuli for the wing surface were light touch or puffs of air. Cutaneous receptive fields were centered around Haarscheiben (Pinkus domes) which are found in a prominent array on both ventral and dorsal surfaces. These raised dome structures are aligned in single file rows which form a distinctive geometric pattern across the wing. This pattern is consistent within any one species but varies between species. Histological examination reveals identifiable Merkel cells within these Haarscheiben.

These findings reveal a highly specialized somatosensory system in which peripheral receptors are of a single type (Haarscheibe) arranged in an elaborate geometrical array. The central representation of these receptors consists of a segregated population of slowly adapting units. The importance of this system to the bat is suggested by the relative size of the representation in somatosensory cortex and the preserved representation of both dorsal and ventral wing surfaces. The unique anatomical and physiological features of this system appear well suited to provide specialized sensory feedback from the wing, e.g. to indicate changes in air flow or pressure across its surfaces during flight.

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16.1 DEVELOPMENT OF THE AUDITORY ORGANS OF THE BULLFROG: A LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDY. W.P. Shofner and A.S. Feng. Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801

We have previously demonstrated that during development, physiological changes occur in the peripheral auditory system of the bullfrog (Shofner and Feng, 1981). In particular, the distribution of best excitatory frequencies of intermediate and high frequency selective primary auditory fibers shift downward. The changes in frequency selectivity of these two populations of auditory fibers during development presumably reflect in part morphological changes in the amphibian and basilar papillae. The development of these two auditory organs was investigated using light and scanning electron microscopy.

Otic capsules of anesthetized adult (13-17 cm snout -vent length) and juvenile (3-4.5 cm snout -vent length) bullfrogs were opened and gently perfused with buffered 4% glutaraldehyde. Following fixation, entire otic capsules were decalcified, dehydrated, cleared and embedded in paraffin. Serial sections (15 u) were stained in hematoxylin and eosin for light microscopy. For scanning electron microscopy, the auditory organs were excised following fixation and subsequently dehydrated, critical point dried and mounted on aluminum stubs. The mounted specimans were sputtered coated with Au/Pd. Quantitative measurements were made from serial reconstructions of camera lucida drawings of hematoxylin and eosin stained sections and stereopair scanning electron micrographs.

During development, there is a dramatic increase in the volume of the basilar papilla lumen. In addition, the tectorial membranes of the basilar and amphibian papillae increase in size during this period of development. These changes are accompanied by increases in the size of the sensory epithelia and number of hair cells. The morphological changes observed in the basilar and amphibian papillae presumably alter the frequency selectivities of these organs during development.

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16.2 ULTRASTRUCTURAL DEVELOPMENT OF THE EAR IN THE TOADFISH, OPSANUS TAU. Bernd Sokolowski* (SPON: A. B. Butler), Department of Anatomy, Georgetown University, Washington 20007.

Fishes are the only vertebrates in which inner ear hair cells appear to proliferate throughout life. Consequently, fishes are a potential model system for understanding the mechanisms involved in hair cell growth and regeneration in vertebrates. However, since previous studies of the teleost ear have dealt solely with the function and morphology of the adult sensory epithelium, little is actually known about the development of the ultrastructure of these sensory cells. Thus, before we can gain further insights into the mechanisms involved in hair cell proliferation in the adult we need to learn about the ontogeny of the sensory epithelium, particularly with regard to hair cell development and the formation of the different hair cell orientation patterns.

In the present study, ultrastructural development of the saccular macula of the toadfish, Opsanus tau, was studied in the adult and at early ages from an embryo to two weeks post-hatching, using scanning and transmission EM. The adult toadfish saccule has a typical teleost four quadrant hair cell orientation pattern with two vertically oriented and two horizontally oriented hair cell groups. TEM of embryonic tissue shows that the centriole precursors to the kinocilia are present at the 12 somite stage, while kinociliary buds are seen by the 25 somite stage. The initial kinociliary buds are centrally located on the surface of the sensory cell and are seen prior to the development of the stereocilia. The saccular sensory epithelium in the one-day post-hatched animal (approximately 10-12 days post-fertilization) has cells with a single kinocilium, as well as other cells with a kinocilium and a few small stereocilia. Cells with only a kinocilium are of two types. One type has a kinociliary bud that is centrally located on the apical portion of the cell while the other has a more developed kinocilium located to one side. The fully developed hair cell ciliary bundles are first encountered at one end of the developing saccular macula and as growth continues they are found over the whole epithelial surface. Individual sensory cells appear to have their adult orientation as soon as their kinocilium and stereocilia are recognizable. This is particularly noticeable by 7 days post-hatching. By 10 to 14 days post-hatching, the shape of the saccular sensory epithelium approximates that of the adult. At these ages there are a large number of hair cell ciliary bundles over the entire surface of the macula although bundles are still forming. (Supported by NSF grant BNS 80-0943 and by a grant from the Lerner-Gray Fund for Marine Research of the American Museum of Natural History).

16.3 POSTNATAL DEVELOPMENT OF SPIRAL GANGLION CELLS IN THE RAT COCHLEA A.M. Schwartz, M. Parakkal*, and R.L. Gulley. NIH, NINCDS-LNO, Bethesda, MD 20205.

The postnatal development of the spiral ganglion in the albino rat was studied using light and electron microscopy. The morphological characteristics distinguishing type 1 from type 2 spiral ganglion cells were defined and the critical period for distinguishing the two types of cells was identified. Twenty-eight OM strain rats divided into eight age groups (newborn,2,4,6,8, 10,14, and 30 postnatal days) were used. At birth, the spiral ganglion consists of a homogenous population of small, densely packed spherical cells, which have large nuclear/cytoplasmic ratios. The cell somas are surrounded by a single layer of glia. No synapses were observed on the spiral ganglion cell bodies at this or any subsequent age. During the first postnatal week, the cells mature slowly. The spiral ganglion cell exhibits numerous filopodial extensions of the membrane, and the developing myelin extends long glial fingers which can be seen wrapped around the filopodia. By the end of the first postnatal week, the myelin sheath generally consists of a few layers of loose myelin. By postnatal day 8, the two types of spiral ganglion cells can be distinguished. The type 2 cells are often found in clusters of two or three and are usually, but not exclusively,located at the periphery of the ganglion. Using phase contrast light microscopy, on toluidine blue stained semithin sections, the type 2 cell is smaller than the type 1. Its cytoplasm stains less intensely compared with the type 1 cell, while its nucleus often appears darker with a fine rim of dense material along its edge. In the electron microscope, the most characteristic features of the type 2 cells are the dense packing of neurofilaments but marked sparsity of other cytoplasmic organelles, especially rough endoplasmic reticulum. Type 2 cells generally are ensheathed by multiple layers of loose myelin that even retain small amounts of glial cytoplasm in the lamellae, while type 1 cells have compact myelin surrounding the soma. By day 14, spiral ganglion cells are morphologically mature based on cell size, packing density, nuclear/cytoplasmic ratio, lack of filopodia, and full complement of cytoplasmic organelles. The myelin sheath, however, continues to thicken after this age. The results are in good temporal agreement with previously observed findings on the morphological maturation of the organ of Corti and the electrophysiological development of the auditory system in the rat.

16.4 THE ORGANIZATION OF THE SACCULE OF HELOSTOMA TEMINCKI. William M. Saidel* and Arthur N. Popper, Department of Anatomy, Georgetown University, Washington, D.C. 20007

The complex structure of the saccule of H. temincki (the kissing gourami) was investigated with several techniques. The hair cell orientation pattern was studied with SEM; the nerve innervation pattern, by the reduced silver stain of Winkelmann and Schmitt; acoustic frequency mapping by intense sound destruction of hair cells; and electrophysiological performance by microphonic and axon recordings. The results suggest that this saccule is organized along a 'pseudo place-like' mechanism.

Three types of hair cells were seen with SEM. Hair cells with a long kinocilium and a few, short stereocilia border the sensory epithelium. The bulk of the epithelium is covered by hair cells with a graded ciliary bundle. A type intermediate in structure is found between these two hair cell types. The hair cells are distributed into four orientation groups, the anterior two are oriented along the fish's horizontal axis, and the posterior two, along the fish's vertical axis.

The reduced silver preparations revealed a pattern of innervation onto the saccular sensory epithelium by eighth nerve fibers that followed certain rules: i) the anterior saccular branch innervates only the horizontally oriented cells and the posterior branches innervate the vertically oriented cells; ii) fibers appear to innervate hair cells of specific types; and iii) fibers may individually innervate hair cells in a single orientation group or opposite orientation groups. Innervation of hair cells in orthogonally polarized orientation groups has not been seen.

Use of intense tonal stimulation demonstrated that pure sine wave stimuli destroy hair cells in large and reproducable loci on the saccular sensory epithelium. The distribution of frequency specific loci is not linearly tonotopic. Support for frequency responsive loci has also been derived from microphonic recordings and responses of single axons known to innervate specific epithelial regions.

This organization is simultaneously useful in providing input for sound localization, and in the analysis of the acoustic signals to the fish. We propose that the response of the hair cells on the saccular epithelium will be dictated by the motions of the otolith relative to the epithelium. This motion will be in the form of an 'orbit' within the saccular chamber due to the modes of stimulation of the teleost ear, and that each 'orbital' will depend upon the characteristics (i.e., direction, spectral components) of the stimulating signal.

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16.5 SIMILAR NUMBERS OF STEREOCILIA ON BOTH LARGE AND SMALL APICAL SURFACES OF HAIR CELLS IN THE GOLDFISH VESTIBULAR SYSTEM.
C. Platt and J. D'Andrea*. Dept. Biological Sciences, Univ. Southern California, Los Angeles, California 90007.

Fishes, unlike mammals, have only vestibular Type II cylindrical hair cells as sensory receptors in their inner ears. Yet this single type exhibits wide diversity in the form of the ciliary bundle that extends from the apical surface of each cell. Each bundle consists of one true cilium, the kinocilium, at one end of an array of several stereocilia. Since the recent work of Hudspeth and co-workers has shown that the stereocilia mediate the mechanosensory transduction process, we wanted to examine the numbers of stereocilia present in some of the different bundle forms.

The utricle is the gravistatic otolith organ of the ear, and has a dished sensory macula. Anteriorly, a region called the striola contains large hair cells that have much larger apical surfaces and thicker, taller ciliary bundles than those found in the floor of the macula, the cotillus. After conventional aldehyde fixation and osmium post-fixation of goldfish inner ear tissues, we used a sonication bath for 5-30 sec to shear off the ciliary bundles from the utricular maculae. After dehydration and critical-point drying, scanning electron microscopy then showed the base of each bundle as an array of dots on the apical surfaces of the sensory cells. We measured the length, width and stereociliary number of the arrays on large cells sampled from the striola and small cells from the cotillus of utricles from 6 goldfish.

The mean lengths of the arrays are roughly 1.5 μm for the small bundles and 2.0 μm for the large bundles; the mean widths of the arrays are roughly 0.8 μm for the small and 1.2 μm for the large bundles. The differences between small and large are significant, for both lengths and widths. If the area covered by the array is approximated by a rectangle, the small arrays sampled occupy a mean area of less than 1.5 μm^2, but the large arrays occupy roughly 2.5 μm^2. Surprisingly, the stereociliary number found in both large and small arrays was close to 40, the small cells averaging roughly 38 and the large cells roughly 43.

These results show that the large cells have almost twice the spatial area of the stereociliary array on their surfaces, but only about 10% more stereocilia in that array than the small cells. If the stereociliary number is important to the magnitude of the receptor potential, this similarity suggests that the small cells may be as mechanosensitive as the large cells. If this difference partly represents growth, it suggests that many small cells already have a full complement of cilia, which then thicken and spread apart slightly during development.

16.6 MOTION OF HAIR-CELL STEREOCILIA IN THE AUDITORY RECEPTOR ORGAN OF THE ALLIGATOR LIZARD.
T. Holton* and A. J. Hudspeth (SPON: M. E. Olds). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

In vertebrate auditory organs, hair cells are thought to transduce mechanical displacements of their hair bundles into receptor potentials. In the auditory receptor organ of the alligator lizard, the basilar papilla, hair cells rest on a basilar membrane which moves in response to sound. The motion of this membrane differs from that of the mammalian membrane in that it is not tonotopically organized; that is, the frequency selectivity of motion does not vary with longitudinal position along the organ (Peake, W. T. and Ling, A. L., J. Acoust. Soc. Am., 67:1736, 1980). However, responses of cochlear-nerve fibers innervating the basal portion of the papilla are tonotopically organized (Weiss, T. F., Peake, W. T., Ling, A. L. and Holton, T., Evoked Electrical Activity in the Auditory Nervous System (Naunton, R. F. and Fernandez, C., eds.), Academic Press, 1978, p. 91). In this region, the hair bundles protrude into fluid unencumbered by overlying tectorial structures and have heights that vary monotonically with position along the organ. We have sought to determine whether tonotopic organization exists at the mechanical input to the hair cells, that is, whether hair bundles in different positions along the organ move maximally in response to stimuli of different frequencies.

We have observed the motion of individual hair bundles in response to mechanical stimulation in an in vitro preparation of the lizard's basilar papilla. The organ is cemented across a perforated partition separating two fluids, a high-K^+ saline bathing the top (hair-bundle) surface of the organ and a high-Na^+ saline bathing the bottom (basilar-membrane) surface. Motion of the organ, induced by driving the fluid in the bottom compartment with a piezoelectric bimorph element, is observed under differential-interference-contrast optics with xenon-flash stroboscopic illumination and recorded on 35mm film or videotape.

The main finding is that the motions of the distal tip of a hair bundle and of the top surface of its hair-cell body are different, frequency-dependent functions of longitudinal position. As a result, the relative motion between these structures, which constitutes the effective mechanical stimulus to the cell, is frequency-selective and tonotopically organized: the longest hair bundles move preferentially at low frequencies (∿ 1.5 kHz), while the shortest hair bundles move preferentially at high frequencies (∿ 4 kHz). Thus, tonotopic organization of hair-bundle motion and neural response are correlated, suggesting that receptor-organ micromechanics partly determines such neural response properties as frequency selectivity and tonotopic organization.

16.7 MECHANICAL PROPERTIES OF HAIR BUNDLES OF RECEPTOR CELLS IN THE GUINEA PIG COCHLEA.
D. Strelioff and Å. Flock*, Division of Head and Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024 and Dept. of Physiology II, Karolinska Institutet, S-104 01 Stockholm, Sweden.

The static mechanical properties of hair bundles (HB) on sensory hair cells were investigated and the stiffness of the HB were measured in an isolated preparation of the guinea pig cochlea. The preparation consisted of about three-quarters of a coil of the second (T2), third (T3) or fourth (T4) cochlear turn which was carefully dissected free and maintained in tissue culture medium (Leibowitz, L15) at room temperature. Removal of the stria vascularis and the tectorial membrane permitted access to the apical surfaces of the hair cells. The coil was mounted in a microscope chamber where it was held horizontally and could be rotated to provide proper alignment of the HB with respect to a probe of known stiffness. The tips of the stereocilia of a hair cell and the probe were viewed with differential interference contrast optics while the probe, a vertical quartz glass fiber about 1 μm in diameter and 500-700 μm in length, was used to displace the row of stereocilia by 1 μm. From measurements of the bending of the probe, the stiffness of each HB was determined.

It was found that the cilia within each HB are attached to one another, that the cilia do not bend but pivot at their attachment to the hair cell and that both attachments of the cilia are irreversibly damaged by horizontal deflections greater than 2 μm (≈ 25°). The HB stiffness (defined as the force required to deflect the tips of the HB by 1 μm) was 0.90±0.35 dynes/cm for the first row of outer hair cells in T4, increased toward the base of the cochlea (3.48±0.38 dynes/cm for the first row of outer hair cells in T2) and decreased radially for the three rows of outer hair cells. On the basis of measurements of the lengths of the hair bundles we suggest that the longitudinal variation in stiffness is due mainly to the decrease in the length of the HB from apex to base. Another significant finding was that the HB are approximately twice as stiff in the excitatory direction (toward the basal body) as in the opposite direction.

The longitudinal stiffness variation may play an important role in the tuning properties of the cochlear partition. In addition, the asymmetric stiffness may account for some of the nonlinear properties of cochlear mechanics, receptor potentials and neural responses recorded in the mammalian auditory system.

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16.8 MOTION OF BASILAR PAPILLA AND HAIR CELL STEREOCILIA IN THE EXCISED COCHLEA OF THE ALLIGATOR LIZARD: RELATION TO FREQUENCY ANALYSIS.
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The basilar papilla in the alligator lizard is an elongated auditory receptor organ containing hair cells attached to the basilar membrane which vibrates in response to sound. The axis of symmetry of the stereociliary bundle of each hair cell - presumably the sensitive direction for displacement - is perpendicular to the long axis of the papilla. CF's of nerve fibers are between 0.2 and 4 kHz and vary systematically with location in the nerve (Weiss et al in Evoked Electrical Activity in the Auditory Nervous System, Naunton and Fernandez, eds., 91, 1978). Yet measurements of basilar membrane velocity suggest that the frequency response does not vary from one part of the basilar membrane to another and thus does not provide a basis for the observed variation of nerve fiber CF (Peake and Ling, J. Acoust. Soc. Am. 67: 1736, 1980). Where then does frequency analysis take place? One possibility is that, within the basilar papilla, modes of motion may occur which are not evident in the measurements of basilar membrane motion. It has also been suggested that the gradual variation in maximum length of the free-standing stereocilia in the posterior two-thirds of the organ, from 30 μm in the 1 kHz region to 12 μm in the 4 kHz region, may provide a micromechanical basis for frequency analysis over this range of CF (Weiss et al, loc cit).

To address these issues, the motion of the basilar papilla and of the free-standing hair cell stereocilia that results from acoustic stimulation has been observed under a microscope. The cochlear duct was excised and the basilar papilla with its supporting limbus was mounted in a drop of lizard Ringer's solution across an opening in an air-filled chamber; the organ was stimulated with tones (0.2 - 6 kHz; 110 - 130 dB SPL) and viewed stroboscopically at 400 - 800X. Results are: (1) the papilla rocks about an axis parallel to its length in the direction of symmetry of the stereociliary bundles, i.e. in the direction of sensitivity of the hair cells; (2) the papilla moves in phase along its entire length, consistent with Peake and Ling's findings; (3) above 3 kHz, the displacement of the high-CF region exceeds that of the rest of the papilla, perhaps providing a partial basis for frequency analysis; (4) the stereociliary bundles move relative to the underlying cuticular surface in the direction of presumed hair cell sensitivity; the phase angle of the relative movement of the stereocilia tips is a function of frequency and of the position of the hair cell along the basilar papilla; these findings are consistent with an explanation of frequency analysis in the papilla based on length-dependent mechanical tuning of stereociliary bundles.

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16.9 THE LEAKY INTEGRATOR MODEL OF THE SPIKE GENERATION MECHANISM SIMULATES THE BEHAVIOR OF UTRICULAR AFFERENTS. E. Soto*, R. Budelli*, M. T. González-Estrada* (SPON: M. M. Rodríguez-Budelli). Unidad de Investigaciones Cerebrales, Instituto Nacional de Neurología y Neurocirugía. México, D. F. and Universidad Autónoma de Puebla. Puebla, México.

The leaky integrator model simulates the phase-locking of the discharges from several sensory afferents. There is a group of utricular afferents (Type I from Budelli & Macadar, 1979) which shows phase-locking; so, these afferents can be simulated by a leaky integrator. Another group of afferents (Type II) does not show phase-locking, so it might be assumed that the behavior of these afferents can not be simulated by the leaky integrator. Nevertheless we would expect from a model to reproduce the behavior of every type of afferents by an adequate selection of the parameters. The leaky integrator always shows phase locking for stimulus with frequencies closed to the spontaneous firing rate and small intensities. There is a chance that if certain model parameters are chosen, the range of frequencies within which phase-locking is apparent can be very small. This would reduce the probability of finding phase-locking experimentally. We were able to determine the frequencies and amplitudes range within which the phase-locking is produced for different time constants of the model. We found that, for values of the time constant (τ) similar or shorter than the period (ρ) of the spontaneous firing, the range of the stimulus parameters where the model produces phase-locking is relatively wide, as it is observed for Type I afferents. For $\tau >> \rho$ the range vanishes giving a result similar to that of Type II afferents. If we select the parameters in a way such that the model produces phase-locking, it, also, reproduces the relation frequency of stimulus-rate of firing and the gain curves of Type I afferents. On the other hand, if we select the parameters in order not to produce phase-locking, the model reproduces the curves of Type II afferents. This shows that quantitative changes in the time constant of the model produce qualitative changes in the behavior of the simulated afferents.

Budelli & Macadar (1979): Statoacoustic properties of utricular afferents. J. Neurophysiol. 42:1479.

16.10 EFFERENT MODULATION OF HAIR CELL TUNING IN THE TURTLE COCHLEA. J.J. Art*, A.C. Crawford*, R. Fettiplace* and P.A. Fuchs. Physiological Lab., Cambridge Univ., Cambridge CB2 3EG, England.

Most major features of frequency selectivity of turtle hair cells can be described in terms of a parallel resonance which correctly predicts their responses to acoustic stimuli and current injection (Crawford, A.C. & Fettiplace, R., J. Physiol. 306:79, 1980; ibid, J. Physiol. 312:377, 1981). This frequency selectivity is decreased following efferent stimulation. Intracellular records from hair cells show that there is a direct synaptic input onto these cells which controls both their mean membrane potential and their tuning properties.

A long-lasting hyperpolarization (50-300 ms) is recorded from hair cells following electrical stimulation of the vestibular branch of the eighth nerve. In response to a single shock the inhibitory post-synaptic potential (IPSP) has a rise time of about 25 ms and decays exponentially with a time constant of about 50 ms. The amplitude of the IPSP facilitates strongly with multiple shocks so that eight shocks separated by 4 ms may produce IPSPs which are as large as 25 millivolts in cells with resting potentials of between -45 and -58 millivolts. When hair cells are hyperpolarized by injecting currents in excess of -300 pA, the IPSP is reversed in polarity, indicating that the synaptic potential arises from an increase in membrane conductance to ions whose equilibrium potential is approximately 30 mV negative to the resting potential.

The evidence for a damping of the hair cell resonance during the efferent IPSP is as follows: a. a decrease by 1.6-25 fold in the amplitude of the receptor potential at the best or characteristic frequency (CF); b. a broadening of the frequency selectivity of the filter as measured by an increase in the half-power bandwidth of the linear tuning curve with little change in the CF; c. a faster decay of the oscillations in hair cell membrane potential in response to acoustic clicks or steps of injected current.

We reported previously a large efferent inhibition and resultant loss of frequency selectivity in the auditory afferents of this species (Art, et al., J. Acoust. Soc. Am. 69:S52, 1981). We believe that the combined effect in the hair cells of the long lasting hyperpolarization and the frequency selective desensitization following efferent stimulation is sufficient to account for both of these observations.

16.11 IMMUNOCYTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE-LIKE IMMUNOREACTIVITY IN OLIVOCOCHLEAR FIBERS IN THE GUINEA PIG COCHLEA. J. Fex, R.A. Altschuler, M.H. Parakkal*, and F. Eckenstein*. Lab Neuro-otolaryngology, N.I.H., Bethesda, MD 20205 and Max-Planck-Institute, D-8033 Martinsried, FRG.

There is considerable biochemical, physiological and pharmacological evidence that olivocochlear efferent fibers are cholinergic. With the recent reorganization of this system into lateral and medial components (Warr 1980), it has become obvious that most of this evidence pertains only to the medial component ending primarily by outer hair cells. In this study we used immunocytochemical techniques to determine the localization of choline acetyltransferase (ChAT)-like immunoreactivity in the guinea pig cochlea. Choline acetyltransferase serves as an excellent marker for cholinergic neurons, fibers and terminals.

The indirect immunofluorescence technique of Coons and the PAP technique of Sternberger were both utilized on cryostat sections through the cochlea and on whole cochlea with the bony shell removed, respectively. Antiserum to ChAT from pig brain, raised in mouse and antiserum to a ChAT-mouse IgG complex, raised in rabbit were used in this study. Both gave identical results. ChAT-like immunoreactivity was seen in the inner spiral bundle, tunnel spiral bundle and by the bases of inner hair cells, corresponding to the lateral system of efferents, and in tunnel crossing fibers and patches by the bases of outer hair cells corresponding to the medial system of efferents. This indicates that both systems of olivocochlear efferents are cholinergic. We have previously demonstrated enkephalin-like immunoreactivity in olivocochlear fibers. Studies are now in progress to determine if there is co-localization of ChAT and enkephalin-like immunoreactivities.

16.12 MULTIPLE RESERVOIR MODEL OF NEUROTRANSMITTER RELEASE BY A COCHLEAR HAIR CELL, H.A. Schwid* and C.D. Geisler. Department of Neurophysiology and of Electrical and Computer Engineering, University of Wisconsin-Madison, Madison, Wisconsin 53706.

A probabilistic model is described for transmitter release from cochlear hair cells and for primary auditory neuron EPSP's and discharge patterns. The present model assumes that several reservoirs of neurotransmitter exist, having individual probability-of-release functions centered at successively higher stimulus intensities. Refilling of the reservoirs, a crucial process, is not uniform but is from the highest intensity reservoir downwards. The model accurately mimics the adaptation of successive EPSP amplitudes of the afferent neuron of the goldfish sacculus, and, for mammalian auditory-nerve fibers, the adaptation of neural discharge rate, the saturation of onset and steady-state neural rate versus intensity, and the change in neural rate in response to incremental stimuli. The model also produces realistic interval and period histograms. Contrasting characteristics of previous single-reservoir models will also be presented. The data to be shown support the hypothesis that multiple populations of neurotransmitter are involved in the afferent hair-cell synapses.

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16.13 "NEUROTRANSMITTER SYNTHESIZING ENZYMES IN THE DEVELOPING CHICK INNER EAR: A MODEL FOR CELLULAR LOCALIZATION OF PUTATIVE CHEMICAL MEDIATORS IN THE VESTIBULAR SENSORY PERIPHERY". °G. Meza; °P. Cuadros*, °I. Lopez* and +M. Ruiz*, °Dept. Neurociencias, CIFIC, UNAM and +Dept. Histología, Fac. Medicina, UNAM, México, D.F.

Neurotransmission in the sensory periphery of the vestibular system is chemical in nature; the efferent neurotransmitter is apparently acetylcholine; strong evidence favors GABA as the afferent mediator. We have reported that glutamate decarboxylase (GAD), the GABA synthesizing enzyme, is present in a homogenate of isolated chick vestibular cristae and took it as evidence for GABA participation in the system; however, we were not able to say in which cells this activity was located. The chick vestibular embryogenesis is well known: the sensory epithelium develops prior to the arrival of nervous fibers. If afferent neurotransmission were GABAergic, GAD activity would be found and measurable in hair cells at a stage in embryogenesis before the fibers arrive to it; if acetylcholine were the efferent neurotransmitter its synthesizing enzyme, cholineacetyltransferase (CAT) should be detectable when efferent fibers reach the neuroepithelium. We investigated these possibilities and give the results obtained. Homogenates of vestibular cristae from chick embryos of 13,17,19 days, of the day of hatching and of 1-day old chicks were obtained and GAD and CAT activities were measured. Morphological features were followed by light and EM microscopy. We found that in the 13-day old embryo, GAD activity was already a 60% of that present in the 1 day-old chick and it reached the level of the latter at the day of hatching; whereas CAT activity in the earlier stage studied was barely detectable increasing slowly along development. The morphological studies showed that hair cells, as early as in the 13-day old embryo present some of the characteristics of the mature cell i.e., hair tufts in the apical part; typical afferent and efferent connections were not present at this stage but appeared slowly as ontogenesis proceeded. These results show that GAD activity is detectable in vestibular cristae of chick embryo before the sensory epithelium receives any connections implying that this activity is located in a cell type of this structure most probably the hair cell. On the other hand, the activity of CAT is measurable later in development in coincidence with the arrival of efferent fibers; thus CAT activity must be located in efferent terminals.

Although additional evidences are needed to proof the cellular source of the neurotransmitter candidates in the vestibular sensory periphery these results altogether can be taken to support the GABAergic nature of afferent neurotransmission and suggest that most probably the efferent mediator is acetylcholine.

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16.14 ULTRASTRUCTURAL STUDY OF DEVELOPMENT OF SYNAPTIC ORGANIZATION IN THE VESTIBULAR SYSTEM OF THE CHICKEN. K. D. Peusner. Dept. of Anatomy, Geo. Washington Univ. Sch. of Med., Washington, D.C. 20037.

The tangential nucleus comprises a part of the avian lateral vestibular complex in the medulla. The embryonic bodies of the principal cells of the nucleus are contacted by large vestibular nerve afferents, the colossal fibers, by means of very large spoon-shaped synapses in a one-to-one relationship. Previous ultrastructural studies showed that the spoons attain their largest size in the late embryo, followed by atrophy postnatally (Peusner, 1980,1981). Concomitantly, small synaptic terminals seemed to increase in numbers that contact the principal cell body.

The current goal was to quantify these developmental changes in synapses. Montages were made at 31,000X magnification of 15 principal cell bodies at each of 3 stages of spoon transformation: at 15 embryonic days (time of onset of gap junctions), at hatching (when vesicular synapses disappear, although gap junctions persist), and at 3 years (when spoons have electron-dense axoplasm). The selection of bodies for the montages was random with regard to the region of the tangential nucleus and the plane of section through the body, but was dependent on the visibility of the entire perimeter of the somata, their adequate preservation, and their contact by a spoon profile.

The amount of linear surface of the somata covered by all types of synaptic terminals is constant at about 55% at all the ages studied. The spoon occupies a diminishing linear surface, on the average, from embryos (40%) to hatchlings (12%), which is retained in 3 year olds (12%). The average length of the spoons in the montages was 27.7 um in embryos (range: 13.7-38.2 um), 7.9 um in hatchlings (range: 1.7-21.1 um), and 9.0 um in 3 year olds (range: 2.6-19.6 um). In contrast, the small synaptic terminals occupy an increasing linear surface, on the average, from embryos (16%) to hatchlings (42%), that persists in 3 year olds (44%). This increase in linear surface covered by small synaptic terminals may be attributed in part to an increase in the average lengths (1.2 um in embryos, 1.6 um in hatchlings, and 2.1 um in 3 year olds) and to a fluctuation in the number of synaptic terminals per 100 um of linear surface (14.3 in embryos, 27.1 in hatchlings, and 21.6 in 3 year olds). In summary, birth marks a time for major developmental changes in synaptic endings in the avian lateral vestibular complex. In hatchlings, spoons have atrophied from their embryonic form. In addition, the surface of the principal cell body in the vicinity of a shrinking spoon is filled in by small synaptic terminals of unknown origins, that allow for a constancy in the amount of somatic surface membrane covered by synaptic profiles. (Supported by USPHS grant 5 R01 NS15633 and 7 R01 NS18108.)

16.15 DISTRIBUTION OF SENSITIVITY VECTORS IN CENTRAL VESTIBULAR UNITS RESPONDING TO LINEAR ACCELERATION, N. Daunton and G.Melvill Jones. Biomedical Res. Div., NASA Ames Res. Centre, Calif. 94035 and AMRU, Dept. of Physiol., McGill Univ., Montreal, PQ, Canada.

A population of 639 neural units responding to natural vestibular stimulation has been examined in 26 decerebrate cats by conventional extracellular microelectrode recording in a brainstem region deemed likely to receive primary afferent inputs from the otolith organs (W. Mehler). The animals were located in a stereotaxic device (22° head down) mounted on a platform suspended from the ceiling by either three parallel springs (for vertical stimuli), or three parallel steel cables (for horizontal stimuli). All units were first tested 'audibly' for qualitative assessment of response to 3-D rotational and linear acceleration. 304 units (48%) responded to rotation, but not linear acceleration, of which 171 were selectively horizontal canal dependent (91 Type I, 80 Type II), 89 vertical canal dependent (76 Type I, 13 Type II) and the remainder (44) received a variety of convergent canal inputs. Of those responding to linear acceleration (52%), 81 units were successfully recorded and averaged during 60 cycles of sinusoidal oscillation in each of the three orthogonal x (side-to-side), y (fore-aft) and z (up+, down-) directions, at 0.15 'g' amp. and 0.59 Hz. The magnitude and direction of individual resultant sensitivity vectors were subsequently calculated with the object of mapping their polar distribution relative to the skull. Mean 'spontaneous' activity in the stationary animal was 12.3 (SE 1.2) AP/sec, with no significant differences in subpopulations responding primarily in x, y and z directions; nor between those excited in +z and -z directions. The latter finding implies a neural bias opposing the DC effects of gravity on the peripheral otolith organs. There was no correlation between resting level and neural gain (r=0.06). Mean sensitivity (neural gain) of resultant vectors was 196 AP/sec/g (SE 19; range 25-1150) the only significant segregation being between those excited by contralateral (227±26 AP/sec/g) and ipsilateral (111±15) acceleration along the x axis. The directions of sensitivity vectors were not clustered along the x, y, z axes. Rather, when projected on the x-y (horizontal) plane they tended to concentrate along diagonal axes close to the planes of the vertical canals. Additional concentrations were found close to the horizontal plane. These directional trends suggest that central representation of both rotational and linear vestibular information tends to be coordinated within the same three orthogonal planes, namely those of the canals. In line with this is the fact that those 'otolith' units which selectively received H-canal input, had linear sensitivity vectors oriented close to the horizontal plane.

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16.16 EVOKED FIELD POTENTIALS MEASURED IN THE VESTIBULAR NUCLEI BEFORE AND AFTER UNILATERAL PARTIAL LABYRINTHECTOMY. Janet F. Ott. Dept. Biol., Univ. Southern Calif., Los Angeles, CA. 90007.

To test for possible physiological changes involved in compensation, the plastic process allowing recovery of function after ablation of the utricle and semicircular canals unilaterally, evoked field potentials were measured in the vestibular nuclei ipsi- and contralateral to the remaining ear. Two - four goldfish each were tested at one day, two weeks, and two months post-op, in addition to several control fish having both ears. Recordings were made in lightly anesthetized fish with exposed utricles.

Recordings were made on the surface of the brain along the midline from the fourth ventricle to the rostral edge of the vagal lobes every 200 microns. At the place of the highest amplitude response, a lateral series was recorded at right angles to the midline on the ipsilateral (stimulated) side. Response amplitudes increased, then decreased with distance from the midline. At the highest amplitude response, a depth profile was made recording again at every 200 microns. Except in two cases, this was the point where a unique waveform change was noted at some particular depth. Recordings were then made at points 200 microns rostral, caudal, medial and lateral to this point and at the corresponding points on the contralateral side.

A point of unique waveform change was always noted on the ipsilateral side, generally directly beneath the point of highest response amplitude on the surface. Points surrounding the unique waveform had responses that increased, then decreased in amplitude with depth, but showed no change in waveform. These ipsilateral responses do not change after unilateral partial labyrinthectomy. However, the contralateral side shows changes. Control and day one fish show a small response on the surface at the point corresponding to the unique waveform approximately one-tenth to one-fourth the size of the ipsilateral surface wave. There is no change in waveform with depth. Beginning at approximately two weeks, the surface waveform increases in amplitude and the waveform changes with depth corresponding to the change on the ipsilateral side. This change is not seen in all two week post-op fish and may be related to the fact that the behavior is variable during this period also (Ott, 1981). At two months, all fish show some change on the contralateral side. This is similar to findings by Dieringer and Precht in the frog (1979). This work was supported by a Grants-in-Aid for Research by Sigma Xi.

16.17 OTOACOUSTIC EMISSIONS IN MAN AND DOG: ASSOCIATION WITH COCHLEAR PATHOLOGY. M.A. Ruggero, B. Kramek* and N.C. Rich*. Depts. of Otolaryngology and Veterinary Clinical Sciences, Univ. of Minnesota, Minneapolis, MN 55455.

Spontaneous and evoked oto-acoustic emissions (OAEs) are reported to be common in seemingly normal-hearing humans. However, cases have been noted where spontaneous OAEs (SOAEs) existed at frequencies near which audiometry indicated hearing deficits. We present here data on one human ear and one dog ear, both of which produce SOAEs. In both cases, the SOAE is associated with audiometric abnormalities. Review of our data and the related literature has led us to formulate a simple hypothesis to explain the origin of both spontaneous and impulsively evoked OAEs; we propose that both phenomena arise from sharp spatial transitions in the organ of Corti, between areas of outer hair cell (OHC) loss or other pathology, and areas of relative normality.

The first author's right ear produces a SOAE at 7529 Hz and 16 dB SPL. An external continuous tone is able to suppress the SOAE. The 3 dB iso-suppression curve is broadly tuned and displaced, relative to the SOAE, toward higher frequencies. Impulsive stimuli (clicks and short tone pips) are able to synchronize the SOAE. In contrast to the suppressive effect of continuous tones, impulsive stimuli can synchronize the SOAE only if they possess sufficient energy at the SOAE frequency. An audiogram notch exists at frequencies just below that of the SOAE.

An intense SOAE (9000 Hz, 47 dB SPL) has been recorded from one ear of a young dog. Its suppression tuning curve is broadly tuned and displaced toward lower frequencies. BSER audiometry indicates severe high frequency hearing loss, sharply sloping toward normality near the SOAE frequency.

We explain the occurrence of both SOAEs and impulsively evoked OAEs in terms of disruption of active feedback mechanisms of the OHCs upon basilar membrane vibration. Each OHC feeds back positively upon its segment of basilar membrane and negatively upon adjacent segments. Normally, the two feedbacks largely cancel each other, leaving a small positive effect: the "stimulus frequency acoustic emissions" demonstrated by Kemp and Chum (in Studies in Hearing, van den Brink and Bilsen, eds., 1980). However, if a patch of OHC loss exists, adjacent segments will be released from the negative feedback and will respond to an impulsive stimulus with exaggerated oscillations at their resonance frequencies, thus producing OAEs. At particularly sharp transitions between normal and abnormal regions of the organ of Corti SOAEs will be generated. (Supported by NIH Grant NS12125)

16.18 RIPPLE NOISE PROCESSING BY THE GOLDFISH AUDITORY SYSTEM. R.R. Fay*, W.A. Yost* and S. Coombs. Parmly Hearing Institute, Loyola University of Chicago, Chicago, IL 60626.

Goldfish were classically conditioned to discriminate between complex acoustic signals called rippled noise. Ripple noise produces a pitch sensation for man and has been used to describe the filtering properties of the auditory periphery. This signal may be produced by taking a broadband noise with a flat power spectrum, delaying it (t sec) and adding it back to its undelayed version, resulting in another broadband noise with a "rippled" power spectrum -- power varying sinusoidally with frequency. The frequency spacing between spectral peaks and the spectral location of the first peak is equal to the reciprocal of the delay (t^{-1} Hz). As t increases, the spacing between peaks decreases, resulting in increasing ripple densities.

The minimum detectable change in spectral spacing (Δt^{-1}) was measured for rippled noises with spectral peak spacings at 800, 400, 200, and 100 Hz (t values of 1.25, 2.5, 5 and 10 msec). Results from 5 animals showed that the smallest detectable spacing (Δt^{-1}) remained approximately constant at about 5% of t^{-1}. These results are in close agreement with those from humans (Yost et al, J. Acoust. Soc. Am. 63: 1166, 1978), despite large differences between humans and fish in the mechanical filtering properties of the auditory periphery. These data will be compared to other psychophysical results using ripple noise as well as to neurophysiological findings on the processing of ripple noise by single units in the eighth nerve of the goldfish. These results will be discussed in terms of the temporal and spectral mechanisms responsible for the processing of this complex stimulus. (Work supported by NIH and NSF).

16.19 EXAMINATION OF THE NEURAL NATURE OF THE FREQUENCY FOLLOWING RESPONSE IN CATS. R.L. Snyder and C. Schreiner, Epstein Lab., University of California, San Francisco, CA. 94143.

Two different sets of sustained AC signals can be recorded from the auditory system in response to low frequency (below 4kHz) tones. The first is the well characterized cochlear microphonic (CM), which is generated by structures in the cochlea--primarily outer hair cells. The second is the less well defined frequency following response (FFR), whose generators are presumably neurons central to the cochlea. The FFR, usually recorded with scalp electrodes, is often dismissed as a remote recording of the CM. However, in the course of an examination of the FFR, we have been presented with a number of properties of the FFR which clearly separate it from the CM.

In anesthetized cats a platinum-iridium ball electrode was placed at the edge of the round window, and a pair of similar electrodes placed straddling the auditory nerve as it exits the internal meatus. In addition silver wire electrodes were inserted through the skin at the vertex and below each pinna. The CM was recorded differentially from the round window electrode and one of the electrodes at the internal meatus. The FFR was recorded either by recording differentially via the the auditory nerve electrodes (AN-FFR) or via the skin electrodes (S-FFR).

The intensity-response (I/O) function, the frequency-response (transfer) function, the adaptation properties, and the influence of masking stimuli were examined in the CM, the AN-FFR, and the S-FFR. The amplitude of the CM was a linear function of the input stimulus intensity up to high levels (110 dB SPL) and its phase was relatively constant. The amplitude of the AN-FFR and the S-FFR saturated or showed a marked inflection at moderate stimulus intensities (70-90 dB SPL). The phase of the FFR's was constant and different from the phase of the CM except after the inflection where it approximated that of the CM. In the transfer function the amplitude of the FFR's was relatively larger at low frequencies (below 2kHz) than the comparable CM. Moreover, the FFR transfer function displayed the expected interference dips at higher intensity levels which were unseen in the CM transfer function. In the averaged response to tone bursts the CM showed no signs of adaptation or an offset response. In contrast the FFR's showed marked adapatation and a pronounced offset response. The time course of the FFR adaptation closely matches the time course of neural adaptation seen in the auditory nerve. Non-simultaneous masking has no effect upon the CM, while forward pure-tone maskers profoundly supress the FFR's. Finally, section of the auditory nerve or injection of TTX into the cochlea greatly diminish the FFR's, while they leave the CM unaffected or only slightly decreased.

From these results we conclude that the FFR at low to moderate stimulus levels is largely a neural response whose generators are central to the cochlea and separate from those for the CM. cochlea. (Supported by the Deafness Research Foundation and a NASA grant #NCA2-OR665-003).

17.1 SYNAPTIC GAIN CONTROL IN INSECT PHOTORECEPTORS.
S. R. Shaw* (SPON: I. A. Meinertzhagen). Psychology Dept., Dalhousie University, Halifax, N.S., Canada B3H 4J1.

The first synaptic relay in the insect visual pathway is the site of amplification of small signals from photoreceptors, and of gain control during light adaptation. Despite the availability of a complete qualitative description of neural connections in the columns (cartridges) of the synaptic zone in Diptera (flies), it is unclear how gain control is exercised. Large field potentials complicate intracellular recording from the synaptic zone,or lamina, which lies inside a part of the blood-brain barrier.

To overcome field potential contamination, responses from the receptor terminal have been monitored intracellularly back in the cell body, by antidromic cable conduction up the axon. Responses from the other terminals in a cartridge then become accessible, via electrical connections between next neighbors, established by multiple gap junctions (Shaw, S.R. & Stowe, S., J. Cell Sci. 53, 115-141, 1982). A single fiber optic probe moved to the appropriate facet lens stimulates each terminal individually, because each originates from a cell under a different facet. The attenuated terminal response so recorded discloses a fast transient wave of inhibitory feedback, starting just after the direct photoresponse. Thus a brief surge of high gain afferent output can escape from the terminal, before suppression follows. The polarity of the feedback signal recorded in the soma, and the disposition of diffusion barriers around the cartridge, now rule out direct electrical interaction between pre- and postsynaptic cells as an explanation for the feedback. Instead, chemical synaptic action seems to be responsible. This is local in origin, since feedback is centered spatially upon the terminal stimulated. In addition, the total loop delay (1.5-2.0 msec, which has to include the two chemical synapses), leaves negligible time for synaptic processing outside the cartridge itself.

Two particular follower neurones make reciprocal connections back to receptor terminals within the same cartridge, and are well placed to originate the feedback. Quantitative counts of the numbers of their synapses revealed an initially perplexing result. Neither neurone comes close to matching the number of reciprocal synapses driving it directly from the receptors (~200 per terminal), seeming not to indicate a powerful gain control system. However, when the known amplification of the primary afferent synapse is taken into account, only a small percentage of the number of afferent synapses is needed in the feedback arm of the circuit, to obtain total transient suppression of receptor output. Correctly balancing the relative numbers must be an important function of the developmental program, in a system like this using high gain synapses. (Supported by NSERC grant A9593).

17.2 RPE BASAL MEMBRANE HYPERPOLARIZATION FOLLOWS LIGHT-EVOKED, K^+-DEPENDENT, APICAL MEMBRANE HYPERPOLARIZATION. Edwin R. Griff* & Roy H. Steinberg. Depts. of Physiol. & Ophthalmol., Univ. of Calif., San Francisco, CA 94143.

The response of vertebrate photoreceptors to light leads to a decrease in $[K^+]$ in the subretinal space between the neural retina and the retinal pigment epithelium (RPE). This decrease in $[K^+]$ hyperpolarizes the RPE apical membrane and, therefore, increases the trans-epithelial potential. We have recently observed in gecko retina that the subsequent recovery (decrease) of the trans-epithelial potential is not caused solely by a repolarization of the apical membrane, but rather by a hyperpolarization of the RPE basal membrane. In the cat, a similar sequence of RPE membrane potential changes occurs. Thus, absorption of light by photoreceptors leads to a hyperpolarization of the RPE apical membrane followed by a hyperpolarization of the basal membrane.

We have further studied this basal event in the perfused, isolated RPE of gecko. We mimicked the light-evoked K^+ decrease by changing $[K^+]$ in the apical bathing solution. Decreasing $[K^+]ap$ from 5 to 2 mM first hyperpolarized the apical membrane, and this was followed by a hyperpolarization of the basal membrane. The basal membrane response was more easily studied by increasing $[K^+]ap$. Increasing $[K^+]ap$ from 2 to 5 mM depolarized the apical membrane and this was followed by a depolarization of the basal membrane. Increasing $[K^+]ap$ from 5 to 20 mM, however, depolarized only the apical membrane. Addition of ouabain to the apical solution caused a depolarization of the apical membrane but no subsequent basal response. Similar results were obtained by increasing apical $[Mg^{++}]$. Thus, the basal membrane event was not triggered solely by polarizing the apical membrane. The basal depolarization produced by increasing $[K^+]ap$ from 2 to 5 mM was blocked by replacing apical $[Cl^-]$ with isethionate or methyl sulfate, suggesting that K^+ must enter the cell to produce the basal depolarization. We propose that the interaction between changes in $[K^+]ap$ and the response of the basal membrane involves a change in intracellular $[K^+]$.

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17.3 EVIDENCE THAT LOCALIZED LASER-PRODUCED LESIONS CAN RETARD THE PROGRESS OF RETINA DEGENERATION IN RCS RATS.
D. Bowyer*, M.M. Behbehani, J. Ruffalo & G. Kranias* (Spon: G. Khodadad) Dept. of Physiology, Dept. of Anatomy and Dept. of Ophthalmology. Un. Cincinnati Coll. Med., Cincinnati, OH 45267.

RCS rats develop a degenerative disease that begins at age 19 days and progesses rapidly, causing blindness by age 2 months. These animals have been used as a model for retinitis pigmentosa. It has been shown that in these animals the phagocytosis of the rod outer segment disks by the pigment epithelium is abnormal. Since laser-produced lesions of the retina can increase phagocytosis, we tried to find out if laser lesions can retard the progress of retina degeneration. 19-day old RCS rats that were kept in total darkness from birth were anesthetized by morphine and fitted with contact lenses. 500 micron laser spots at 100 mw were used to produce 12 lesions in the retina of one eye. The other eye was used as control. Electroretinograms (ERG) were recorded from these animals at weekly intervals, beginning one week after the lesion. When the ERG recorded from the normal and the lesioned eye showed a significant difference, both eyes were removed and prepared for histological examination. The results of these experiments showed that the ERG recordings from the lesioned side display a prominent "a" wave for up to three weeks after the "a" wave of the ERG of the non-lesioned side has been abolished. Histological examination showed that there is an increase in number of phagosomes in the laser-lesioned retina as compared to the non-lesioned side. Since the persistence of the "a" wave in the ERG is an indication of the function of the photoreceptors, these studies indicate that the progress of the retina degeneration can be retarded by laser surgery. (Supported by a grant from the Ohio Lions)

17.4 LIGHT ADAPTATION OF ROD AND CONE PATHWAYS IN THE GOLDFISH. Robert Paul Malchow and Stephen Yazulla. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

The sensitivity changes of cells in the outer retina of the goldfish produced by steady, monochromatic backgrounds were investigated using an isolated retinal preparation. The extracellularly recorded, aspartate-isolated PIII response was used to monitor the responses from rods and cones. Intracellular techniques were used to examine the responses of rod horizontal cells, which contact only rods, and H1 horizontal cells, which receive input predominantly from red cones. Response vs. intensity (RvI) curves were generated using hyperbolic tangent functions as used by Naka and Rushton (1966), followed by the construction of increment threshold curves. Test flashes consisted of 100ms, monochromatic wide field light and were substituted for backgrounds. Responses were first obtained from the dark adapted preparation, followed by backgrounds near threshold intensity, which were then increased in 0.5 log unit increments.

Backgrounds just above threshold caused a compression of the PIII RvI curve, with more intense backgrounds resulting in a lateral shift along the intensity axis. A clear rod-cone break in the increment threshold curve was not apparent. The rod contribution to the increment threshold curve was obtained by isolating dark adapted goldfish retinas at midnight, a procedure by which all cone outer segments are stripped away with the pigment epithelium while leaving the rods intact. Such a preparation yields a PIII response that has a normal dark adapted sensitivity. Background illuminations now result primarily in response compression of the PIII, with a complete suppression of response at higher backgrounds. Rod horizontal cells also adapt to background illuminations by a compression of their RvI curves, with a concommittant narrowing of the curve's width, and intense backgrounds also lead to complete response suppression. H1 horizontal cells have a higher absolute threshold than the PIII or rod horizontal cells and have a much broader response range in the dark. Moderate backgrounds cause a response compression of these cells, as well as a narrowing of their response range. More intense backgrounds result in a lateral shift of these cells' RvI curves along the intensity axis.

This study, comparing light adaptation of rod and cone pathways in the distal retina of a single species, has shown how the rod system approaches saturation as background illumination becomes more intense, while the cone pathway remains able to respond by laterally shifting its RvI curve along the intensity axis. We are currently examining the convergence of these pathways at the level of the mixed rod-cone bipolar cells.

This study was supported by NIH Grant EYO 1682.

17.5 A SPECIES COMPARISON OF PEANUT LECTIN BINDING TO SPECIFIC PHOTORECEPTOR CELLS AND SYNAPTIC REGIONS IN THE RETINA. J. C. Blanks and L. V. Johnson*. Doheny Eye Foundation and Departments of Ophthalmology and Anatomy, University of Southern California, Los Angeles, CA 90033.

Although lectins have been used to study surface oligosaccharides of rod and cone photoreceptor cells in intact retinas and dissociated retinal cells, no lectin has been reported that binds preferentially to cones versus rods. We have found that application of FITC-conjugated peanut lectin (PNA) to cryostat sections of mouse retina results in binding that appears to be confined to a subpopulation of photoreceptor cells. PNA has carbohydrate binding activity highly specific for D-galactose-B-(1→3)-N-acetyl-D-galactosamine. The developmental appearance of PNA binding in postnatal mouse retinas coincides with the appearance of the outer synaptic layer (OSL) and the formation of photoreceptor inner and outer segments. PNA binding within the OSL appears in discrete patches, possibly associated with large synaptic pedicles of cone photoreceptor cells.

To further investigate the possibility that PNA binds specifically to cones, we have extended our study to include the cone dominant retinas of goldfish and chicken. Additional studies of PNA binding in rabbit, monkey and human all suggest that, indeed, PNA binding is specific for cone photoreceptor cells. To obtain optimum morphology, the tissue was fixed in 4% paraformaldehyde, embedded in acrylamide, frozen and cryostat sections were cut at 6-8 um. After application of FITC-PNA to the sections, binding could be detected associated with some photoreceptor cell bodies and cellular processes, that often could be traced to the patchy fluorescent areas in the OSL.

Electron microscopic investigations are underway to establish the identity of PNA-positive photoreceptor cells and specific regions of synaptic layers, as are biochemical characterizations of PNA-binding molecules in the retina.

17.6 ULTRASTRUCTURAL STUDIES OF SYNAPSES IN THE OUTER PLEXIFORM LAYER OF RETINAS OF WILD TYPE (C57BL/6J +/+) AND PEARL MUTANT (C57BL/6J pe/pe) MICE. M.A. Williams*, J. Gherson*, L.J. Fisher, L.H. Pinto. Department of Biological Sciences, Purdue Univ., W. Lafayette, IND. 47907, and Department of Ophthalmology, Henry Ford Hospital, Detroit, MI 48202

The synaptic zone of outer plexiform layer of eight mice was examined for the number and anatomical characteristics of the ribbon synapses. Three of the animals were pearl mutants and five of the animals were wild type.

Electron-dense, bulbous thickenings with diameters ranging from circa 100 to 240 nanometers were found in association with the ribbon lamellae. The electron-dense thickenings were either swellings of the ribbon lamellae near one end of the ribbon, or were discrete electron-dense spheres of similar dimensions near the ribbons. The thickenings were found only in rod spherules. Some spherules contained as many as three separate electron-dense spheres. Eleven per-cent of the ribbons in the retinas of the pearl mutants had associated swellings (247 ribbons examined). Less than 0.6% of the ribbons from the wild-type animals had associated swellings (498 ribbons examined). The spherules which contained swellings were often seen clustered throughout the retina in discrete foci of three to four neighboring or nearly-neighboring terminals.

All animals were light-adapted, anesthetized with ether, perfused intracardially with formaldehyde and glutaraldehyde, and the eye-cups processed for electron microscopy. The animals had been on a twelve hour light/twelve hour dark cycle and were sacrificed between 1100 and 1400 hours.

A similar nodular enlargement of the synaptic ribbon of rods has been reported in retinas of albino rats after excessive exposure to bright fluorescent light (Kuwabara, T. and M. Funahashi, 1976). The accompanying extensive mitochondrial damage that was observed in these rats however, was not present in the pearl retinas.

These anatomical observations suggest that the bulbous swellings may be related to the demonstrated sensitivity deficit of the pearl visual pathway, (Balkema et al, 1981).

17.7 THE GUINEA PIG RETINA CONTAINS BOTH MET- AND LEU-ENKEPHALIN. Judith L. Mosinger*, Douglas W. Hoffman and Joanne C. Gutierrez*. [SPON: M. Martin]. LNO, NINCDS, NIH, Bldg. 36, Rm. 5D-32, Bethesda, MD 20205.

This laboratory has recently published the first report of enkephalin-like immunoreactivity in a mammalian retina (Altschuler, Mosinger, Hoffman and Parakkal, PNAS, 79: 2398, 1982). Enkephalin-like immmunoreactivity has previously been reported in the non-mammalian vertebrate retina (Brecha, et. al. PNAS, 76: 3010, 1979; Eldred and Karten, Soc. Neurosci. Abstr., 7: 277, 1981). In all cases this immunoreactivity has been confined to amacrine cells and their processes in the inner plexiform layer. However, enkephalin-like immunoreactivity has not been seen in other mammalian species examined; e.g., the albino (OM) rat. To identify the molecular forms of the enkephalins in retina, extracts of guinea pig retina were analyzed by high pressure liquid chromatography (HPLC) and radioimmunoassay (RIA) for content of enkephalin-related peptides.

Retinal sonicate was centrifuged, and the supernatant injected directly onto a Waters microBondapak octadecylsilane (10 micron) column equipped with a guard column filled with C_{18}/Corasil. Fractions were isocratically eluted with 0.05% trifluoroacetic acid in 25% acetonitrile at 0.6 ml/min. One min. fractions were collected in a liquid N_2 bath, and then lyophilized to dryness. Samples were reconstituted in a phosphate-BSA buffer for RIA. RIA's were performed using two commercially available antisera raised against met-enkephalin, demonstrating significant cross-reactivity to leu-enkephalin. The use of these antisera coupled to these HPLC conditions permitted the assay of both met- and leu-enkephalin in one short (20 min.) chromatographic run and subsequent immunoassay.

Both met- and leu-enkephalin were found in the guinea pig retina, as was a third substance, which was neither met- nor leu-enkephalin but shared immunochemical characteristics with both. The identity of this third immunoreactivity moiety is not known, but it behaved chromatographically in the same manner as the enkephalin-immunoreactive substance seen in guinea pig cochlea and hippocampus, in which enkephalins are also found.

17.8 SYNAPTIC CONTACTS OF ENKEPHALINERGIC AMACRINE CELLS IN THE RETINA OF TURTLE (PSEUDEMYS SCRIPTA). W.D.Eldred and H.J.Karten. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, New York 11794

Antiserum directed against enkephalin (Chang, Burroughs-Wellcome) labels specific bistratified amacrine cells in the retina of turtle. We have examined these labeled amacrine cells (outside the visual streak) at the ultrastructural level to determine the nature and location of their synaptic contacts and the intracellular localization of enkephalin-like immunoreactivity (ELI) within these neurons. Throughout the cytoplasm there was diffuse DAB reaction product which coated the external surfaces of all organelles. The labeled neuronal profiles contained three classes of vesicles: 1) numerous small unlabeled clear-core vesicles (60 nm in dia.) which were clustered at conventional chemical synaptic release sites; 2) scattered large unlabeled dense-core vesicles (130 nm in dia.); and 3) scattered large labeled vesicles (130 nm in dia.) filled with a core of electron-dense reaction product.

These bistratified amacrine cells with ELI arborize in two strata within the inner plexiform layer (IPL); one wide stratum in the proximal IPL and a second narrow stratum in the distal IPL near the inner nuclear layer. Amacrine cells with ELI receive conventional chemical synaptic contacts from other unlabeled amacrine cells and ribbon synaptic contacts from unlabeled bipolar cells in both the proximal and distal strata of the IPL. In the proximal stratum of the IPL, amacrines with ELI make conventional chemical synaptic contacts onto unlabeled cell processes which lack synaptic vesicles and are probably from ganglion cells. Small unlabeled clear-core synaptic vesicles are clustered at these release sites. Our results suggest that enkephalin-like substances co-exist with at least one other neurotransmitter in these neurons. These bistratified amacrine cells integrate information from bipolar and amacrine cells in both the proximal and distal IPL and relay information to ganglion cells in the proximal IPL. Physiological studies are needed to determine the significance of the synaptic contacts in visual processing. This research supported by EY03801 to WDE and by EY02146 to HJK.

17.9 **BAR SYNAPSES ARE MADE BY THE INTERSTITIAL AMACRINE CELL OF THE CICHLID FISH RETINA,** **R. P. Zimmerman, Departments of Neurological Sciences and Physiology, Rush University, Chicago, IL 60612.**

The inner plexiform layer (IPL) of the retina of the cichlid fish *Astronotus ocellatus* has been shown to have an orderly three-dimensional structure (Hibbard,E.,*Exp.Eye Res.*,*12*:175,1971; Zimmerman, R.P.,*Invest.Ophthalmol. Vis.Sci. 22(Suppl.)*:112, 1982). The IPL is divided into approximately equal thirds by proximal and distal grids of neuronal processes. A roughly square pattern is imposed by the array of radial processes of the Mueller glial cells which run perpendicular to the plane of the retina.

Of particular interest is an unusual type of amacrine cell which has its cell body and processes restricted to a narrow 10 - 15 µm sublamina within the proximal grid. These *INTERSTITIAL AMACRINE CELLS* (following the terminology used by Cajal for the mammalian retina) have a triangular cell body 20 µm long and 10 - 12 µm in diameter at the widest point. The three primary processes of the interstitial amacrine (IA) branch at angles of 90 - 100 degrees; all branches remain within the plane of the sublamina.

The interstitial amacrine cell of the *Astronotus* retina is the first reported instance of processes of an identified class of amacrine cell in the vertebrate retina containing presynaptic dense specializations. The IA's contain dense presynaptic bars which are approximately cylindrical, about 60 nm in diameter and 0.22 - 0.75 µm in length. This presynaptic bar is often opposed by two postsynaptic profiles in a dyad arrangement. The postsynaptic components of the dyad may be a bipolar cell and an amacrine cell process, or two amacrine cell processes. IA's receive direct synaptic input from bipolar cell terminals as well as from other classes of amacrine cells, and are presynaptic to bipolar terminals (at reciprocal synapses), amacrine cells and ganglion cell dendrites. In addition, IA's make large gap junctions with adjacent IA's, but not with any other class of retinal cells. These gap junctions suggest the possibility of electrical coupling between IA's.

17.10 DISTRIBUTION AND MORPHOLOGY OF DOPAMINERGIC NEURONS IN THE RETINA Clyde W. Oyster, Nicholas C. Brecha and Ellen S. Takahashi. School of Optometry, University of Alabama in Birmingham and Center for Ulcer Research and Education, UCLA.

Dopaminergic neurons in the retinas of several species have been labeled by an immunohistochemical method employing antisera directed to tyrosine hydroxylase. The peroxidase-antiperoxidase technique, in modified form, shows the complete dendritic morphology of the immunoreactive neurons in flat-mounted retinas.

In rabbit retina, the dopaminergic neurons are amacrine cells. Their somata are confined to the inner nuclear layer and the dendrites ramify in the outer portion of the inner plexiform layer. The cells are sparsely and non-randomly distributed. In spite of the low density (19 cells/mm^2), the dendritic fields overlap extensively; each retinal point lies within at least three dendritic fields.

The dopaminergic neurons in pigeon retina are also amacrine cells; their morphology is quite similar to those in rabbit retina. In the cat retina, however, some of the dopaminergic amacrine cells have somata in the ganglion cell layer. These displaced amacrine cells give rise to a single process which extends up to stratum 1 of the inner plexiform layer. Here, the dendrites of the displaced amacrines ramify along with those of the main amacrine cell population. Even when these two amacrine cell populations are considered separately, however, the dendritic overlap is still significant.

This and other studies have found dopaminergic neurons in the retinas of all species which have been examined. The type of dopaminergic neuron varies between species, but the various dopamine systems share some general characteristics; they are composed of low density, non-randomly distributed cells having extensive, sparsely branched dendrites.

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17.11 SYNAPTIC ORGANIZATION OF DOPAMINERGIC INTERPLEXIFORM CELLS IN GOLDFISH RETINA: AN EM-IMMUNOCYTOCHEMICAL/AUTORADIOGRAPHIC ANALYSIS. Charles Zucker and Stephen Yazulla, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Long Island, NY 11794.

Evidence suggests that in goldfish, the spatial properties of horizontal and bipolar cells are modified by dopaminergic interplexiform cell(DA-IPC) input. All synaptic inputs to DA-IPCs are located in the inner plexiform layer(IPL), while output occurs in the outer plexiform layer (OPL) as well as in the IPL. In this study, this synaptic organization was analyzed by combining EM-immunocytochemical localization of the biosynthetic enzyme tyrosine hydroxylase (TOH) with autoradiographic locolization of ^{3}H-GABA and ^{3}H-Glycine as well as the nicotinic- and muscarinic-cholinergic ligands ^{125}I-α Bungarotoxin (αBTX) and ^{3}H-Propylbenzilylcholine mustard (PBCM). Following one hour of glutaraldehyde and 18 hours of paraformaldehyde fixation at a pH of 10.4, sodium borohydride and buffered ethanol treatments were used to restore antigenicity and allow immunologic reagents to penetrate throughout tissue blocks. Comparison of labeling pattern of anti-TOH with other studies using either the Falck-Hillarp method or the accumulation of the biogenic amine precurser 5,6-dihydroxytryptamine indicates that we are selectively labeling the DA-IPC. DA-IPC cell bodies are found among those of amacrine cells in the proximal inner nuclear layer and send processes to both plexiform layers forming dense plexes in the OPL and the most proximal IPL and a plexus of much lower density in the distal IPL. DA-IPCs make synaptic contact with amacrine cell processes, rarely with bipolar terminals and never with other DA-IPCs. GABA accumulating pyriform Ab amacrine cells ramify in the proximal IPL making many synaptic contacts with bipolar and amacrine cells and frequent en-passant contacts with labeled DA-IPC processes. GABA accumulating processes are also seen contacting the DA-IPCs in the distal IPL. Binding of ^{125}I-αBTx indicates that the DA-IPC receives no nicotinic input. Studies utilizing ^{3}H-PBCM and ^{3}H-Glycine are presently under way. In the OPL, DA-IPCs make numerous synaptic contacts with GABA accumulating H1 horizontal cells and bipolar cells. Similar contacts, not previously described, are also made onto type H2 and H3 cone horizontal cells. The double labeling technique we have employed is a powerful tool to study the geometry of an identified neuron in regard to its specific transmitter inputs. These data when correlated with electrophysiological studies provide much needed information about the synaptic interactions involved in receptive field formation and modulation.

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17.12 AMACRINES RECIPROCAL TO THE ROD BIPOLAR IN CAT RETINA ARE GABA-ACCUMULATING. M.A. Freed* and P. Sterling, Dept. Anatomy, School of Medicine, Univ. of Pennsylvania, Philadelphia, PA 19104.

At least four types of amacrine accumulate exogenous ^{3}H-GABA, but specific circuits involving these neurons have not been identified. We have examined the rod bipolar axon terminal, identified by its dark cytoplasm and unbranched, scalloped form, to learn whether any of its amacrine inputs are GABA-accumulating. In each of several cats one eye was injected with 100uCi ^{3}H-GABA; the animal was perfused 3.5 hours later with a buffered mixture of aldehydes and the retina prepared for EM autoradiography. From a series of 110 consecutive EM autoradiograms we identified three classes of amacrine varicosity associated with the rod bipolar. The AII amacrine, identified by its large mitochondrion, absence of synaptic vesicles, and presence of ribbon contact from the rod bipolar, was non-specifically labeled (0.03±0.02 grains/um^2) as was a second class (0.08±0.06 grains/um^2) identified by its synaptic input to the rod bipolar and lack of a reciprocal contact. A third class, identified by its reciprocal contacts with the rod bipolar invariably showed specific labeling (0.21± 0.03 grains/um^2).

Two types of amacrine, A_{13} (hyperpolarizing) and A_{17} (depolarizing) have been shown by Kolb and Nelson (Vis. Res., 21: 1625, 1981) to receive ribbon contacts from rod bipolars, and we (McGuire et al., 1982) have determined that every amacrine varicosity with such a ribbon contact is reciprocal. This implies that both A_{13} and A_{17} are reciprocal to the rod bipolar and, therefore, are GABA-accumulating. Indeed, A_{13} resembles morphologically our type 1 GABA-accumulating amacrine and A_{17} resembles our small, spherical GABA-acumulating amacrine (Freed et al., Neurosci. Abstr., 1981). Thus, two types of amacrine with opposite responses to light may both provide GABA-ergic feedback to the rod bipolar axon terminal and constitute a dual mechanism for regulating its sensitivity.

17.13 A GOLGI AND AUTORADIOGRAPHIC STUDY OF (^{3}H)MUSCIMOL-LABELED AMACRINE CELLS IN THE CAT RETINA. R. G. Pourcho and D. J. Goebel*. Dept. of Anatomy, Wayne State Univ., School of Medicine, Detroit, MI. 48201.

Five morphologically distinct subpopulations of neurons located in the amacrine portion of the inner nuclear layer (INL) of cat retina have been shown to label with both (^{3}H)gamma-aminobutyric acid (GABA) and the GABA agonist, (^{3}H)muscimol (Pourcho, R.G., Brain Res., 215:187, 1981). In order to provide further information about the dendritic ramification of these cells within the inner plexiform layer (IPL), Golgi impregnation has been combined with electron microscopic autoradiography. Retinas were preloaded with (^{3}H)muscimol and then processed for Golgi impregnation according to the method of Mariani in Kolb, H. et al. (J. Comp. Neurol., 189:31, 1980). After selected cells were photographed and drawn with a camera lucida, the same cells were thin sectioned for autoradiography. In order to prevent chemographic exposure of the nuclear emulsion by dichromate salts, the sections were covered with a formvar film prior to dipping into emulsion.

This technique has been used to characterize four types of amacrine cell with somas in the INL and one type with its soma displaced to the ganglion cell layer which accumulate (^{3}H)muscimol. The interplexiform cell was also found to be labeled, confirming the finding of Nakamura, Y., et al. (Proc. Nat. Acad. Sci., 77:658, 1980) that this cell in the cat may be GABAergic. Among the amacrine populations were a cell with a dendritic field of less than 100μm whose processes are confined to strata 1 and 2 of the IPL, a cell with a dendritic field of 180μm and branches in strata 2-5, and a cell whose dendrites ramify over an 800μm field in strata 4-5. All of these cells exhibited prominent varicosities on their dendritic processes. Another amacrine which ramified exclusively within stratum 2 was also labeled. The displaced amacrine had varicose processes in strata 3-4 of the IPL. Preliminary electron microscopic analysis of the synaptic relationships of these cells indicates that several participate in reciprocal synapses with various classes of bipolar cells.

The combination of Golgi and autoradiographic techniques offers the opportunity to obtain new information regarding the circuitry of neurochemically characterized cells and should aid in understanding the role of these neurons in retinal function.

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17.14 RETINAL GABA NEURON LABELLING WITH (3H)-ISOGUVACINE IN DIFFERENT SPECIES, E. Agardh and B. Ehinger. Dept of Ophthalmology, University of Lund, Lund, Sweden, S-22185.

Retinas from goldfish, chicken, rat, guinea-pig, rabbit and humans were exposed to (^{3}H)-isoguvacine either by intravitreal injection in vivo or by incubation in a balanced salt solution and the distribution of radioactivity was then studied with autoradiography. The substance labelled a set of presumed amacrine cells in all types of animals. The inner plexiform layer was well demarcated and a variable amount of ganglion cells was marked. In goldfish and chicken, radioactivity could also be seen in horizontal cells, particularly so when the retina had detached from the pigment epithelium. Even 24 hours after an intraocular injection there was a significant amount of radioactivity left in nerve cells. Only little glial labelling could be seen at any time. Since the distribution of labelled neurons was similar to that of GABA neurons and since isoguvacine is a potent GABA agonist, it seems reasonable to presume that (^{3}H)-isoguvacine labels GABA neurons. The uptake is selectively neuronal with strong binding to presumed GABA storage sites.

17.15 EFFECTS OF ASPARTATE AND GLUTAMATE ANALOGUES ÓN AMACRINE AND GANGLION CELLS IN MUDPUPPY RETINA. P.D. Lukasiewicz* and J.S. McReynolds. Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

GABA and glycine have been shown to act as inhibitory transmitters in the inner plexiform layer (IPL) of mudpuppy retina, but the excitatory transmitters in the IPL are still unknown. Here we show the effects of three aspartate/glutamate analogues on transient amacrine cells and ON-center ganglion cells in intracellular recordings from superfused mudpuppy eyecups.

Application of 250 μM 2-amino-4-phosphonobutyric acid (APB) eliminated light-evoked responses in depolarizing bipolar cells and ON-center ganglion cells, as well as the ON response of transient amacrine cells, as reported previously by Slaughter & Miller (Science 211: 182, 1981). When synaptic transmission was blocked by 1 mM cadmium (Cd), APB produced no voltage or conductance changes in either transient amacrine cells or ON--center ganglion cells, although it still caused a hyperpolarization and conductance decrease in depolarizing bipolar cells.

250 μM N-methyl-dl-aspartate (NMDLA) produced a depolarization and conductance increase in both transient amacrine cells and ON-center ganglion cells. When applied in the presence of Cd, NMDLA produced the above effects only in transient amacrine cells.

100 μM kainic acid (KA) produced a depolarization and conductance increase in ON-center ganglion cells, also in the presence of Cd; its effect on transient amacrine cells has not been determined.

These results suggest that transient amacrine cells have receptors activated by NMDLA (KA not yet tested) and that ON-center ganglion cells have receptors activated by KA but not by NMDLA. The findings are consistent with the notion that one or more amino acids related to aspartate or glutamate is used as an excitatory transmitter in the mudpuppy IPL, and that if only one such substance is endogenous its receptor sites are pharmacologically different on transient amacrine cells and ON-center ganglion cells. Neither of these cell types has the kind of glutamate/aspartate receptor site found at the synapse from photoreceptors to depolarizing bipolar cells.

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17.16 DISTRIBUTION OF L-ASPARTATE AND L-GLUTAMATE SYNAPTIC RECEPTORS IN MEMBRANES FROM CHICK RETINA, A.M. López-Colomé and F. Somohano*. Departamento de Neurociencias, Centro de Investigaciones en Fisiología Celular, UNAM; 04510 - México, D.F.

L-Glutamate (Glu) and L-Aspartate (asp) have been considered as strong neurotransmitter candidates in the vertebrate retina. It has been proposed that both amino acids could act as transmitters at photoreceptor cell (PR) synapses, as well as at other excitatory synapses in the inner retina. We have attempted to discriminate between Glu and Asp as the PR transmitter by determining the kinetic and pharmacological characteristics of Asp and Glu receptors measured through the binding of ^{3}H-Asp and ^{3}H-Glu to synaptic membranes from both retinal plexiform layers in conditions reported to expose synaptic receptors (Enna and Snyder (1976), Brain Res, 115:174-179).

As we have previously reported for Glu (López-Colomé (1981), Neurochemical Res, 6: 1019-1033), we found saturable, high affinity ^{3}H-Asp binding in membranes from both plexiform layers which exhibited a K_D=40nM for outer plexiform and K_D=12nM for inner plexiform layer and B_{max}=0.666 and 0.4 pmoles/mg protein respectively. In contrast with ^{3}H-Glu binding which is evenly distributed in both plexiform layers, ^{3}H-Asp receptors were found to concentrate in membranes from outer plexiform layer compared with membranes from whole retina. Binding of ^{3}H-Asp was effectively inhibited by Asp analogues N-methyl-DL-aspartate and DL-alpha-amino-adipate whereas the Glu antagonist glutamate-diethyl ester had no effect.

Kainic acid has been shown to destroy amacrine cells and to a less extent, horizontal and bipolar cells, in the chick retina when injected intravitreally at a dose of 60 nmoles/eye (Morgan, I., and Ingham C.A. (1981) Neurosci Lett, 21: 275-280). When ^{3}H-Asp and ^{3}H-Glu binding was measured in these lesioned retinas, a significant increase in the number of receptors (around 100% over control) was observed for both amino acids, although following a different time course.

Our results indicate the presence of different synaptic receptors for Glu and Asp in both plexiform layers of the chick retina, which can be distinguished by means of kinetic constants, some pharmacological characteristics, and the effect of kainate.

(This work was supported in part by grant PCCBNAL 800800 from CONACYT).

17.17 ULTRASTRUCTURE OF THE INNER NUCLEAR LAYER OF THE RETINA OF XENOPUS LAEVIS LARVAE. George J. Kokoris and Leslie J. Fisher. Neuroscience Program, University of Michigan, Ann Arbor, Mich. 48109, and Dept. Ophthalmology, Henry Ford Hospital, Detroit, Mich. 48202

As part of an ongoing study investigating the patterns of proliferation and differentiation of cells within the inner nuclear layer (INL) of the central retina of Xenopus laevis larvae, an ultrastructural analysis of retinal cell types was undertaken using electron microscopy (EM). Tissue was obtained from larvae at various stages of metamorphosis and processed for EM using gluteraldehyde fixation.

Unlike the adult amphibian retina, cells of the larval INL are not stratified according to type within this structure. Horizontal cells, frequently identified by their flattened oblong nuclei, low nucleus/cytoplasm ratio, abundant microtubules, and clumped Nissl material, are the exception, the position of their nuclei always being at the scleral border of the INL, as in the mature retina.

Bipolar cells tend to have a high nucleus/cytoplasm ratio, the envelope of cytoplasm closely following the contours of the nucleus. Their cytoplasm appears electron-lucent, with few organelles aside from large ovoid mitochondria. Bipolar nuclei tend to be large and spherical, with a moderate density of evenly distributed chromatin.

The perikarya of mature Muller fibers often lie in the median stratum of the INL, with dark-staining processes that insinuate and ramify: horizontally, between other cell types in the INL, and radially, from the level of the photoreceptor nuclei to the vitreal border of the ganglion cell layer. The vitreally directed processes contain numerous filaments arranged in bundles, an abundance of smooth endoplasmic reticulum (SER), and widely distributed glycogen granules. Mitochondria are usually observed in scleral portions of Muller fiber cytoplasm.

Amacrine cells are identified by their electron-dense nuclei, which display frequent cytoplasmic inclusions. Their cytoplasm is also electron-dense, and is distinguished by long rills of SER arranged concentrically about the nucleus. Amacrine cells also extend thick processes, containing mitochondria at their origin and vesicles along their length, into the inner plexiform layer.

In addition, a number of cells that seem to be in various phases of the mitotic cycle, including several cells that appear to include two partially formed nuclei within a single cytoplasmic envelope, were observed. This tentative conclusion awaits confirmation with serial sectioning techniques, but is in accord with our autoradiographic data demonstrating mitotic activity within the INL of the differentiated central retina of larval Xenopus laevis.

17.18 GIANT GANGLION CELLS IN THE SKATE RETINA. P. Witkovsky and W.J. Brunken*. Depts. of Ophthal. and Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016 and Dept. of Anat. Sci., SUNY, Stony Brook, NY 11794.

We have demonstrated the existence of giant ganglion cells in the retina of the skate (Raja, spp.), similar in all respects to those found in the dogfish, Mustelus (Stell & Witkovsky, JCN, 148:1, 1973). Giant ganglion cells are identified on the basis of their extensive, elliptical dendritic arbors (1.0 x 1.5 mm) and their large, polygonal cell bodies, measuring 35-45 µm in the longest axis. These neurons can be divided into three classes on the basis of the location of the perikaryon: superficial (ganglion cell/optic fiber layer); intermediate (within the inner plexiform layer); displaced (inner plexiform/inner nuclear layer border). The dendritic arborizations of each cell class are isoplanar, i.e., confined to a narrow horizontal band of inner plexiform layer adjacent to the perikaryal position. Each class of giant ganglion cell forms an irregular matrix over most of the retinal surface with a cell to cell spacing of about 800 µm.

Retrograde transport of HRP was used to demonstrate that the superficial layers of the optic tectum are at least one site of termination for giant ganglion cell axons. Preliminary electrophysiology measures indicate that the superficial class of giants is of the on-center variety. Data on the synaptic connections of giant ganglion cells will be presented.

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17.19 A MODEL FOR THE EVOLUTION OF CENTRAL RETINAL SPECIALIZATIONS M.H. Rowe and B. Dreher School of Anatomy, Univ. of New South Wales & Department of Anatomy, Univ. of Sydney, Sydney, AUSTRALIA

Retinal specializations subserving high acuity vision involve a potential conflict between two fundamental requirements; the neural requirement of high receptor and post-receptor neuron density in the specialized region, and the optical requirement of an unobstructed light path to the receptors. The fovea, found in a variety of vertebrates, is a structural solution to this problem which involves the displacement of all post-receptor retinal neurons away from the specialized region. However, even in animals lacking a fovea, such as the domestic cat, some of the inner retinal neurons, the ganglion cells, are displaced away from the center of the high acuity specialization in a pattern which may illustrate the early stages of foveal evolution.

Most beta (X) cells near the area centralis (a.c.) of the cat, as seen in whole mount preparations and visualized following HRP injections into one optic tract, have a single primary dendrite. At the center of the a.c. these dendrites typically descend vertically through the inner plexiform layer (IPL) and give rise to a dendritic tree which is directly beneath the soma. For cells between 100 and 700 microns from the center of the a.c., however, most dendrites descend obliquely through the IPL resulting in a dendritic tree which is laterally displaced from the soma by as much as 40-50 microns. For many of these cells, the trajectory of the dendrites is such that the somas are systematically displaced away from the center of the a.c., while for other cells, the direction of the displacement seems random with respect to the location of the a.c.

This balance between random and systematic variation in the pattern of ganglion cell soma displacement is unaffected by monocular deprivation. However, in Siamese cats, although central beta cells with laterally displaced somas are as common as in normal cats, the direction of the displacement is predominantly random. Thus, the degree of randomness in the pattern of ganglion cell soma size displacement can vary between strains of cats and appears to be under fairly direct genetic control.

These observations suggest that early stages of foveal evolution involved a transition from a random to a systematic pattern of dendritic displacement among ganglion cells near the area centralis. Thus the presence of random variation in neural populations may be an adequate substrate for natural selection, and the transition from random to systematic variation in the characteristics of a neural population may be a general mechanism of nervous system evolution.

17.20 MORPHOLOGY OF RETINAL GANGLION CELLS IN A TURTLE, PSEUDEMYS SCRIPTA. E.H. Peterson School of Anatomy, University of New South Wales, Sydney AUSTRALIA

The ganglion cell layer of Pseudemys retina is highly differentiated with a horizontally aligned visual streak and a peak density area in the approximate center of the retina (Peterson & Ulinski, '79). Moreover, there is substantial variation in ganglion cell size as a function of retinal locus and some evidence that ganglion cells of different size have idiosyncratic patterns of retinal distribution (Peterson & Ulinski, '82). In the present study I have begun to characterize the morphology and spatial properties of Pseudemys ganglion cells using Golgi impregnation techniques and retrograde transport of horseradish peroxidase applied to the optic nerve or tract.

A majority of the ganglion cells observed to date can be assigned to one of three groups. Type I cells are the largest ganglion cells with somata in peripheral retina greater than 25um and dendritic field diameters of 700-900um. They show little variation in soma or dendritic field size as a function of distance from the streak; e.g. one cell in or just above the peak density area had a 22um soma with a dendritic spread of 500um. They appear to be monostratified with two variants arborising approximately in strata 1 or strata 5 of the IPL. Type II cells have smaller somata and dendritic fields (17-19um and 250-250um in peripheral retina). They typically have relatively numerous pedunculated appendages on the distal dendrites, and somata which are eccentrically placed within the dendritic field, but their dendrites are displaced randomly with respect to the visual streak. In Nissl material, cells of this same relative size tend to occur in pairs as described for alpha cells in cat retina. Type III cells have a characteristic 'lacy' appearance with dendritic branches having fine beaded appendages which descend through the IPL to terminate in a single, well defined layer. In the periphery, Type III cells have radially symmetrical dendritic fields (200-300 um) and relatively small somata (10-12um). Closer to the streak both somata and dendritic fields are markedly smaller (8-10um; 60-80um) and dendrites are displaced toward the streak. In one retina, two Type III cells were impregnated in or very near the peak density area with somata approximately 150um apart, one above and one below the streak. These cells have narrow dendritic arbors only 20um in width which are displaced sharply toward the streak. Thus dendritic field size in Type III cells decreases by a factor of 10 from peripheral retina to the streak in contrast to Type I cells in which the change with eccentricity is much less marked. These cells appear to correspond to Type B cells of Marchiafava & Weiler ('80) and probably form the small cell peak seen in soma size histograms of Pseudemys retina (Peterson & Ulinski, '82) which is most pronounced in the visual streak.

17.21 A MONOCLONAL ANTIBODY AGAINST RETINAL GANGLION CELLS IN THE MOUSE. U.C. Dräger and C.J. Barnstable. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

Balb/c mice were immunized with the ganglion cell layers of formalin-fixed retinas from C57BL/6J mice. Spleen cells from immune mice were fused with P3-x63 Ag8.653 plasmacytoma cells, and hybrid cells were selected in HAT medium. Supernatants of growing cultures were screened on cryostat sections of formalin-fixed C57 retinas, using indirect immunofluorescence. Antibodies of one of the hybridoma lines that were cloned, R3, stained fiber bundles in the ganglion cell layer and processes in the outer plexiform layer. With Triton X-100 added, a network of fine fibers in the inner plexiform layer and, rarely, the outlines of a cell in the ganglion cell layer became visible. This pattern was indistinguishable from the staining we saw with a known antibody against neurofilaments (Wood, J.N. and B.H. Anderton, Biosci. Rep. 1:263-268, 1982).

In developing retinas R3 bound at the earliest age tested, the day of birth, although the staining was not as bright as in adults. A few cell bodies in the ventricular zone and in the ganglion cell layer were stained, in addition to what appeared to be optic fiber bundles. Several days later cells with long processes at the level of the developing outer plexiform layer became positive.

The spatial distribution of the staining in the adult retina was studied in whole mounts. A tangle of thick, sparsely branching processes with small cell bodies was seen in the outer plexiform layer; this may represent a class of horizontal cells known to be rich in neurofilaments. Most of the processes in the ganglion cell layer appeared to be optic axons: they converged on the optic disk and a few could be seen to emerge from large ganglion cells. The identity of other fibers was not clear: a few in every retina formed a loop with both ends heading into the direction of the optic disk, and an occasional fiber could be seen to give off branches. When the eye was injected with colchicine, the overall intensity of the staining increased and many more ganglion cells became visible on account of dense filamentous material in soma and dendrites. All the stained cells were large in diameter, 18 μm on average, which is about twice the size of the bulk of ganglion cells in the mouse; and 6% were displaced ganglion cells, as compared with 1% in the population overall. They formed a small fraction of all ganglion cells, in the best preparation so far between 2-3%. Whether the distribution of the R3 antigen in ganglion cells correlates with functional or projectional characteristics, similar to the neurofibrillar staining restricted to α-type ganglion cells in the cat, is not clear. (Supported by EY01938 and EY03735).

17.22 SOME SPIKES ARE WORTH MORE THAN OTHERS: CONSEQUENCES OF STIMULUS AND ADAPTATION CONFIGURATION. B. Pöpel* Institute of Physiology, Free University Berlin, W.-Berlin, F.R.G.

Center and periphery of cat retinal ganglion cell receptive fields (RF) were stimulated with white light modulated in time by Gaussian quasi-white noise (GWN). The σ-contrast (σ= s.d. of GWN) was 0.27 and was kept constant throughout the experiments, bandwidth was 100 Hz. The adaptation of the unmodulated RF-part was either dark, low photopic (equal to the GWN-mean) or high photopic. Stimuli were uniform discs of center size and large fields minus the discs. Disc diameters were chosen to produce as far as possible pure center and surround responses as estimated from first order kernels under "mean" adaptation ("medium situation").

Without going into theoretical details (R.Eckhorn and B.Pöpel, Vis. Res. 21,435-443 (1981)) the rate of transinformation, T'(bit/s), is taken as a coupling measure denoting the information transfer of a ganglion cell spike train with respect to the stimulus. Dividing T' by the average discharge rate, $\bar{R}$ (spikes/s), gives a new quantity, $\eta = T'/\bar{R}$ (bit/spike), which yields the information represented by a single spike of this response. η denotes the "economy" of the system in achieving a certain information transfer.

Results reported here refer mainly to brisk sustained ganglion cells because of the better possibility of separating the center and surround mechanisms. Response coupling per se (derived, for example, from T' or the total spectral response power) varies across adaptation in these units and is strongest in the "medium situation".This holds for the majority of on- and off-center cells for both center and peripheral modulation.Also, a substantial part of the average discharge is evoked by the dynamic stimulus.However, in only about 25% of our cells do the changes in discharge rate $\bar{R}$ follow the changes in coupling (i.e.,$\bar{R}$ should not be used to estimate coupling). On the other hand, in off-center cells, the "economy"η of the center responses increases for decreasing adaptation level of the surround, and η of the surround increases for increasing adaptation level of the center.Although η should not be taken as a coupling this result means that the antagonistic inhibition of the unmodulated RF-part tends to suppress more the uncoupled spikes. No such clear-cut result emerged from on-center cells.

In the "medium situation" both coupling and $\bar{R}$ are normally slightly higher for the surround (total surround stimulated!).However,η is larger for the center: with a center stimulus, the single spike carries more information than with a surround stimulus. Finally, a result concerning the brisk sustained (BS)/transient (BT) dichotomy. With diffuse dynamic stimuli, BS-units normally yield weaker coupling and η ($\eta \leq 0.5$) compared to BT-units ($\eta > 0.5$). This supports the idea of BT-units being especially sensitive to luminance changes in time.

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17.23 EFFERENT CONTROL OF PATTERN VISION IN LIMULUS. R. Batra* and R. B. Barlow, Jr. Institute for Sensory Research, Syracuse Univ., Syracuse, NY 13210.

Efferent optic nerve activity from a circadian clock modulates responses of the Limulus lateral eye. Previous studies show that at night the efferent input to the retina changes the structure and sensitivity of retinal photoreceptors. Here we describe the effects of efferent activity on optic nerve responses generated by stationary step patterns of illumination on the retina. One half of the eye was brightly illuminated and the other half dimly illuminated. Optic nerve responses of a single photoreceptor located near the center of the eye were recorded for various positions of the step pattern relative to the photoreceptor.

Lateral inhibition enhances the optic nerve response when the photoreceptor is located in the bright region of the pattern near the edge and depresses the response when the photoreceptor is in the dim region near the edge. These maxima and minima in optic nerve responses correspond to Mach bands: they enhance contrast in the illumination pattern. The width of the Mach band, which reflects that of the underlying inhibitory field, is conveniently measured as the distance between the step and the photoreceptor when the Mach band is half maximum amplitude. During the day the width is about 4 photoreceptor diameters in the antero-posterior direction and 2 photoreceptor diameters in the dorso-ventral direction. These measurements correspond to an inhibitory field extending 6 photoreceptors antero-posteriorly and 3 photoreceptors dorso-ventrally from the center. At night the width of the Mach band does not change significantly, but the optic nerve response increases for fixed levels of excitation. Apparently at night the total strength of lateral inhibition decreases with no corresponding change in the size of the inhibitory field. Also, a second inhibitory process, self-inhibition, appears reduced at night.

In sum, these and other results indicate that the efferent input to the retina at night increases excitatory effects and decreases inhibitory effects. During the day, physiological changes in the retina maintain contrast enhancement under bright light without saturation of optic nerve responses. Thus the retina appears to be adapted for low levels of illumination at night and high levels during the day.

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17.24 TRANSFER FUNCTION BETWEEN RECEPTOR POTENTIAL AMPLITUDE & OPTIC NERVE SPIKE FREQUENCY IN LIMULUS IN DARK & LIGHT ADAPTATION. L. T. Wang* and G. S. Wasserman (SPON: H. B. Nudelman). Dept. of Psychological Sciences, Purdue University, W. Lafayette, IN 47907.

Using one microelectrode inserted into a Limulus retinular cell, simultaneous recordings were made of the receptor potential evoked by light falling on that retinular cell and of the optic nerve action potentials generated in the eccentric cell coupled to that retinular cell. The transfer function for sensory quantity was thereby determined directly at the first postreceptor stage over the entire operating range of the retinular cell from resting potential to saturation; this is a more extended range than previous studies had covered. The transfer function had strong nonlinearities which were discontinuous and it could best be described by a tripartite piecewise linear fit. Light adaptation induced a non-parallel shift of the transfer function.

These properties of the postreceptor transfer function produce a number of differences in the way in which the receptor responds to light (I-V function) in comparison to the way in which the optic nerve fiber responds to light (I-SF function): The I-SF function is both steeper and more sensitive than the I-V function. Similar changes have been seen before using indirect techniques in both invertebrates (Laughlin, 1981) and vertebrates (Green and Powers, 1982). Their interpretation has sometimes been problematic because of the indirect nature of the measurements. The fact that the present results were obtained with a single microelectrode which saw all of the responses at the same time suggests that these differences are genuine. A third effect is that the I-SF function exhibits a greater degree of light adaptation than the I-V function because of the extra light adaptation induced by the postreceptor stage. A fourth effect is that the I-SF function cannot be described by the Naka-Rushton function nor by any continuous function. The sharp discontinuities of the postreceptor transfer function yield corresponding discontinuities in the I-SF function although the latter discontinuities are less noticeable because they are riding on a curvilinear background.

Is postreceptor adaptation mediated by a mechanism that is similar to that which mediates receptor adaptation? The present data show certain phenomenological similarities which might have supported the suggestion that the mechanisms are indeed the same. But important differences exist: In addition to the postreceptor discontinuties, the postreceptor transfer function is expansive at low response levels and compressive at high levels while the receptor function only exhibits a compressive nonlinearity. The effect of this dual nonlinearity is to yield a narrow range of intensities over which small changes in light intensity produce large changes in the sensory signal.

17.25 ANALOGUE MODEL OF THE CHROMATICITY AND LUMINOSITY CHANNELS IN THE GENERALIZED VERTEBRATE CONE RETINA. R. Siminoff. Institut für Physiologie der Freien Universität Berlin, F.R.G.

A prototype of the generalized vertebrate cone retina was built using operational amplifiers to simulate retinal neurons. Each retinal neuron consisted of 2 components, a Summator to add inputs and a Leaky Integrator to simulate the synaptic delay for the output. Inverters and gain controls were used to give the proper magnitude and sign to the output voltage. The dynamic characteristics of the model were determined by the frequency-dependent Leaky Integrators, which are low-pass filters, with the major determining factor being the interplay of antagonistic center and surround field inputs to the bipolar cells (BC). Negative feedback from horizontal cells (HC) to cones and its potentiation by electrical coupling of HCs reinforced the effects of the surround field on decreasing the static levels of tonic ganglion cells (GC), as well as increased the transient ON and OFF phases of phasic GCs. The cone mosaic was simulated by a 25 X 21 grid of 525 phototransistors with color-sensitivity produced by color filters placed in fron to the cones. A trichromatic retina was organized into red unit hexagons (UH) as described in my theoretical paper (Siminoff, J. theor. Biol. 86: 673, 1980). Besides phasic and tonic units, there were color-coded (C) and non-color-coded (L) channels with a full compliment of retinal cells as specified in my theoretical paper. At the HC level, the L-HC acted as the path for the surround field of the L-BC, as well as negative feedback for the cones, while the biphasic C-HCs formed the surround fields for the single and double opponent C-BCs. Color-coded GCs reflected the C-BCs and formed concentric receptive fields with the antagonistic color pairs, red and green or blue and yellow (red plus green). The phasic GCs were formed by the convergence of depolarizing-center (DPBC) and hyperpolarizing-center (HPBC) BCs with the HPBC input having an extra "synaptic delay" and negative feedback reinforced the OFF discharge of the phasic GC. Stray light effects, electrical coupling of like-cones and electrical coupling of like-HCs were simulated by a form of positive feedfoward and along with negative feedback from the L-HCs to the cones played important roles in the development of the spatial and chromatic properties of retinal neurons. The analogue model has a number of physiological correlates. X- and Y- cells are both tonic cells with the X-cells having a minimum of surround field input, while the Y-cells having a strong surround field input. For the C-channel, color-coding was improved by negative feedback and electrical coupling in spite of the sloppy filter characteristics of the cones.

This work was done while the author was a Fellow of the Fritz Thyssen Foundation.

17.26 MODEL OF ELECTRORETINOGRAM b-WAVE GENERATION: TESTING THE K^+ HYPOTHESIS. Eric A. Newman and Louis L. Odette.* Eye Research Institute of Retina Foundation, Boston, MA 02114 and Boston University, Boston, MA 02215.

The K^+ hypothesis of electroretinogram b-wave generation holds that increases in extracellular K^+ concentration ($[K^+]_o$) depolarize Müller cells, leading to the generation of radially directed current flow and a transretinal potential. Several questions have been raised concerning this hypothesis. 1) If $[K^+]_o$ increases drive both Müller cell depolarization and b-wave currents, why is the Müller response much more prolonged than the b-wave? 2) Why do the K^+ ejection experiments of Yanagida and Tomita (1982) lead to intraretinal potential profiles different from those of the b-wave? 3) Why are distal $[K^+]_o$ increases measured in some experimental preparations smaller than those needed to generate the b-wave?

We have investigated these objections to the K^+ hypothesis by simulating b-wave generation with a computer model representing relevent retinal structures. The model incorporates the following components. 1) Two time-dependent K^+ sources representing the light-evoked $[K^+]_o$ increases in the inner and outer plexiform layers, 2) a time and $[K^+]_o$ dependent K^+ sink representing the $[K^+]_o$ decrease in the rod inner segment layer, 3) diffusion of released K^+ through extracellular space, 4) active and passive K^+ re-uptake, 5) spatial variations in the K^+ diffusion coefficient and the volume fraction of extracellular space, 6) an extraretinal shunt resistance. Müller cells are modeled with 1) internal resistance, 2) spatial variations in membrane K^+ permeability, and 3) a membrane potential specified by the Nernst equation and transmembrane current flow. $[K^+]_o$ distributions in time and retinal depth are computed from imposed K^+_o source-sink densities. Based on these computed $[K^+]_o$ distributions, Müller cell potentials, current source-density profiles and intraretinal potentials are calculated.

Imposed $[K^+]_o$ distributions similar to those measured experimentally during the b-wave lead to the generation of a transient b-wave response and a prolonged Müller response in the model system. Current source-density distributions and intraretinal potentials generated by the model match experimental results closely. In addition, the model generates a realistic slow PIII potential in response to prolonged $[K^+]_o$ decreases in the distal retina and reproduces the K^+ ejection results of Yanagida and Tomita accurately. Simulations also suggest that tissue damage caused by K^+-selective micropipettes in experimental preparations may lead to a serious underestimation of the distal $[K^+]_o$ increase. The simulations demonstrate that the spatio-temporal properties of intraretinal b-wave voltages and Müller cell responses can be generated according to the K^+ hypothesis, by passive Müller cell depolarization driven by variations in $[K^+]_o$. Supported in part by N.I.H. grant EY 04077 and N.S.F. grants BNS 801539 and 7824162.

17.27 K^+ SOURCES IN AMPHIBIAN RETINA, C. J. Karwoski, C. Nicholson, & L. M. Proenza. Vision Research Laboratory, Univ. Georgia, GA, and Department of Physiology and Biophysics, NYU Medical Center, NY.

There is presently some question concerning the number and location of light-evoked K^+ sources in the retina, and what role their associated K-increases play in the generation of field potentials, specifically the b-wave. In particular, at the level of the outer plexiform layer, a light-evoked K^+ source (distal K^+ increase) remains an elusive phenomenon. Here, we begin to provide quantitative detail from depth-profiles in frog and mudpuppy retinas.

Two forms of analysis were useful for resolving a distal K^+ increase which, nevertheless, could not be seen in all cases:

(1) Plots of K-increase latency vs. depth show relative minima distally, at the OPL, and proximally, at the IPL. Both distal and proximal K-increases show a strong "surround turndown" -- responses to diffuse stimuli are ∿65% smaller than those to 250 μm spots. Individual K-increases in the distal retina usually reach, at most, only ∿25% of the amplitude that can be observed proximally, while the total distal K-increase is only 5-10% of that seen proximally.

(2) Increases in $[K^+]_o$ are generated by K^+ sources, but the spatial distribution and time course of these sources are obscured because of diffusion. However, a source distribution, $Q(x,t)$, can be computed from knowledge of the time and space derivatives of the concentration by using a diffusion equation that includes the volume fraction (α) and tortuosity (λ) of extracellular space (Nicholson and Phillips, 1981, J. Physiol., 321: 225-257): $Q = \alpha[\partial c/\partial t - (D/\lambda^2)(\partial^2 c/\partial x^2)]$. Such calculations cannot indicate whether K^+ arises from neurons or glial cells, nor can they reveal K^+ sources in equilibrium with K^+ sinks (e.g., clearance mechanisms). Nevertheless, these ion source density calculations are useful and have been initiated in amphibian retinas.

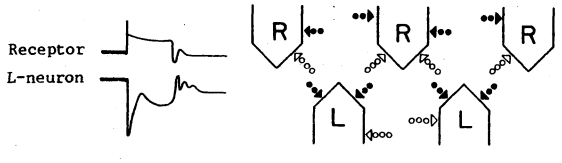
Taken together, these analyses indicate that the profile of light-evoked changes in $[K^+]_o$ consists of: (1) a slow K^+ sink in the photoreceptor layer; (2) a distinct, but small, on-source near the OPL; (3) a large on-source throughout the IPL; (4) an off-source at the IPL, which peaks slightly distal to the on-source; and (5) separate on- and off-sources in the ganglion cell layer. Although evidence for the distal K^+ increase can be obtained from most depth profiles, its amplitude seems too small for it to play the dominant role in b-wave generation.

17.28 LOCAL CIRCUIT FEEDBACK LOOP IN NEURONS OF THE OCELLAR RETINA. Susan L. Stone* and Richard L. Chappell. Dept. of Biological Sciences, Hunter College, New York, N.Y. 10021.

Anatomical[1], electrophysiological and pharmacological studies suggest that synaptic feedback from second order neurons (L-neurons) onto receptor terminals plays a role in transforming the sustained depolarizing response of the photoreceptor into the phasic hyperpolarizing response of the L-neuron in the ocellar retina of the dragonfly. The hyperpolarizing off transient in the receptor response and the depolarizing off transient in the L-neuron are thought to reflect such feedback. Curare eliminated both the L-neuron response and the off transient in the receptor, while picrotoxin modified only the off transients suggesting that the receptor and L-neuron (feedback) transmitters may be acetylcholine and GABA, respectively.[2]

We have recently found evidence which suggests that lateral synaptic interactions between adjacent photoreceptors and L-neuron dendrites may also contribute to the intracellular response waveform of ocellar neurons. Edrophonium, eserine and carbachol reduce the L-neuron light response suggesting that lateral synaptic interactions between photoreceptors may be inhibitory. A conductance increase associated with the depolarizing off transient in the L-neuron indicates that lateral synaptic interactions between L-neurons may be excitatory. The simple model, neglecting such lateral interactions, is no longer sufficient to account for these observations. Consequently, we have developed a more comprehensive model (below). This model is consistent with the original hypothesis that the receptor transmitter (filled circles) may be acetylcholine and the L-neuron transmitter (open circles) might be GABA. According to the model, the receptor transmitter is inhibitory to L-neurons and adjacent receptor terminals, while the L-neuron transmitter has an excitatory effect on the receptor terminals and adjacent L-neuron dendrites. The local circuit is such that a dynamic equilibrium is established at any given light intensity or in the dark, while the response to changes in intensity is enhanced. (Supported by NIH Grant EY-00777.)

[1]Dowling, J.E. and Chappell, R.L. (1972) J. gen. Physiol. 60:121.
[2]Klingman, A.R. and Chappell, R.L. (1978) J. gen. Physiol. 71:157;
Stone, S.L. and Chappell, R.L. (1981) Brain Res. 221:374.



18.1 β-ENDORPHIN MODULATES THE DOPAMINE INHIBITION OF PROLACTIN SECRETION AT THE ANTERIOR PITUITARY. C.Y. Cheung. Div. of Perinatal Biology, Dept. of Physiology, Loma Linda University, School of Medicine, Loma Linda, CA., 92350.

The endogenous opiate β-endorphin (B-EP), has been implicated in the regulation of prolactin secretion. Intravenous administration of B-EP in rats increases prolactin secretion, and the effect is reversible by naloxone. The mechanism by which B-EP stimulates the release of prolactin is not well understood. The present study was undertaken to determine whether B-EP stimulates prolactin secretion by a direct action at the anterior pituitary. Experiments were performed on dispersed anterior pituitary cells in culture. Anterior pituitaries from ovariectomized rats were dispersed using 0.3% collagenase and plated in Ham's F-10 medium containing 15% horse serum and 5% fetal calf serum. At the onset of the experiment the medium was changed to Ham's F-10 containing 20% serum substitute, and the cells were preincubated for 48 hours. The medium was then collected and replaced with fresh medium, or medium containing dopamine, B-EP, or dopamine and B-EP. Ascorbic acid was used to prevent the autooxidation of dopamine, and bacitracin was added to prevent the enzymatic degradation of B-EP. The in vitro effects of B-EP on prolactin release and on the dopamine inhibition of prolactin release were studied over a 24 hour period with sampling of the medium at 1, 3, 5, and 24 hours. The amount of prolactin released into the medium was measured by radioimmunoassay. B-EP at 10^{-6}M, did not significantly alter the release of prolactin into the medium, although small increases of 13%, 15%, 35%, and 17% were observed at 1, 3, 5, and 24 hours respectively. A lower dose of 10^{-8}M B-EP again showed no effect on prolactin release. Dopamine at 10^{-6}M, significantly inhibited prolactin release by 55%, 43%, 48%, and 49% at 1, 3, 5, and 24 hours respectively. However, when the cells were incubated with 10^{-6}M dopamine in the presence of 10^{-6}M B-EP, the inhibitory effects of dopamine at 1, 3, and 5 hours were completely reversed by the opiate. But by 24 hours, the B-EP effect was no longer observed. To investigate whether endogenous B-EP released from the anterior pituitary played a role in maintaining the in vitro release of prolactin, the cells were treated with 10^{-6}M naloxone for 24 hours to block any effects of the opiate before testing with dopamine. Naloxone did not affect the basal release of prolactin, nor the inhibition of prolactin release by 10^{-6}M dopamine up to 24 hours of incubation. Thus, B-EP of pituitary origin may not be released in adequate amounts to affect prolactin secretion from pituitary cells in vitro. The major finding from these studies suggest that although B-EP may not directly stimulate prolactin release from the anterior pituitary, it could modulate the release of prolactin by reversing the inhibitory effects of dopamine.

18.2 EVIDENCE FOR DEGENERATION OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS IN AGING RATS WITH PROLACTIN SECRETING PITUITARY TUMORS, D.K. Sarkar*, P.E. Gottschall* and J. Meites* (SPON: R.A. Bernard), Neuroendocrinology Laboratory, Physiology Department, Michigan State University, East Lansing, Michigan 48824.

The activity of tuberoinfundibular dopaminergic neurons (TIDA) was determined biochemically and with fluorescent histochemistry in young female rats (diestrous, 3-4 mo) and in old rats (anestrous, 24-28 mo) with or without prolactin (PRL) secreting pituitary tumors. Old rats with PRL secreting tumors showed elevated levels of PRL (702±78; n = 19) in the blood as determined by RIA when compared to levels in old (129±12; n = 12), and young (54±7; n = 17) females without tumors. Pituitary weights in situ of rats with tumors (41.4±11.3; n = 11) were significantly higher than in old (12.2±0.5; n = 10) and young (9.2±0.4; n = 16) rats without tumors. Estimation of dopamine content by radioenzymatic assay revealed that there was a significant reduction of dopamine content in the median eminence in rats with tumors (37±2; n = 6), as compared to old (55±4; n = 8) and young (127±22; n = 8) rats without tumors. Dopamine content in the median eminence of old rats also was significantly lower than in the median eminence of young rats. The fluorescent intensity of catecholamines in the external layer of the median eminence was reduced in old animals and the reduction was particularly marked in old females with PRL secreting tumors.

Both tumor and non-tumor bearing old rats showed signs of histological degeneration, as was evident by the presence of punctate autofluorescent lipofusin, and distorted fibers in the arcuate nucleus. These data indicate that there is a loss of dopaminergic neurons during aging and the loss becomes greater as old animals develop spontaneous PRL secreted pituitary tumors. (Aided by NIH research grants AM04784 from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, CA10771 from the National Cancer Institute, and AG00416 from the National Aging Institute to J. Meites).

18.3 SHORT-LOOP NEGATIVE FEEDBACK EFFECT OF PROLACTIN ON THE SUCKLING-INDUCED PROLACTIN RESPONSE AND ON THE PROESTROUS SURGE OF PROLACTIN. Michael Selmanoff, Karen A. Gregerson* and Phyllis M. Wise*. Department of Physiology, Univ. of Maryland, School of Medicine, 660 West Redwood Street, Baltimore, Maryland 21201.

Prolactin (PRL) is thought to exert at the hypothalamus a short-loop negative feedback effect on its own secretion. We tried to block the suckling-induced PRL response and the proestrous PRL surge with sc injections (4mg/kg) of oPRL. The injection of oPRL resulted in the following plasma levels (ng/ml) in these female rats (n=16): Time 0 (undetectable), 0.5h (809±65), 1 (1515±156), 1.5 (2094±200), 2 (2015±248), 3 (1840±231), 4 (958±99), 5 (638±72), 6 (420±42) and 8 (118±40). In 10 day postpartum rats oPRL or vehicle was injected at 6h or 12 and 6h before the onset of suckling at Time 0. Pups were separated from their mothers 4h prior to Time 0 and were allowed to suckle for 40 min. It may be seen from the data that 12 or 6h of prior exposure to high levels of oPRL blunts the suckling-induced rPRL response.

	0'	10	20	30	40	60	90	120
Vehicle	4±	125±	193±	224±	313±	77±	17±	6±
(n=11)	1	35	40	34	23	20	6	2
oPRL-12h	2±	56±	103±	118±	180±	50±	30±	8±
(n=8)	1	19	46	42	47	16	15	3
oPRL-6h	3±	73±	128±	106±	179±	37±	5±	4±
(n=10)	1	21	38	34	37	12	1	1

There is a significant suppression at both 30 and 40 minutes of suckling for both oPRL-treated groups. Considering the area under the curves, the 6h area is 17% less and the 12h area 15% less than controls. In a second experiment in lactating rats (pups removed), oPRL administration was unable to suppress the already low morning basal secretion of rPRL. In 4 day cycling rats, oPRL was injected every 8h starting at 0900h on diestrous day 1. This oPRL treatment totally abolished the proestrous PRL surge and significantly suppressed basal secretion determined in the morning hours.

	0900h	1030	1200	1300	1400	1500	1600	1700	1800	1900
Vehicle	9±	4±	5±	47±	107±	100±	167±	179±	214±	157±
(n=6)	3	1	2	28	60	28	37	46	53	26
oPRL	3±	1±	1±	1±	1±	1±	3±	4±	4±	7±
(n=11)	1	0	0	0	0	1	1	1	1	1

The data indicate that elevated PRL levels dramatically inhibit the hypothalamic mechanism mediating the proestrous PRL surge while having a lesser effect on the mechanism mediating the suckling-induced PRL response. (Supported by NIH grants NS-14611 and AG-02224).

18.4 DIETHYLSTILBESTROL-INDUCED PITUITARY TUMORS: A MODEL FOR THE STUDY OF CONTROL MECHANISMS IN HUMAN PROLACTINOMAS. C. Phelps* and W. C. Hymer*, (SPON: A. Carrillo) Biochemistry Program, The Pennsylvania State University, University Park, PA 16802.

Marked increases in pituitary weight, DNA content and PRL synthesis result from chronic estrogen (DES) treatment in Fischer 344 female rats (Wiklund et al., Endocrinology 109:1700, 1981). In order to further characterize such tumors, we have determined total cell number, PRL content, PRL secretory rates in culture, cell separation, DNA synthetic rates by flow cytometry and effects of subsequent treatment with the dopamine agonist, bromocryptine (BC). Pituitaries from DES-treated (9-10 mg silastic implants) animals increased from 157% at day 8 to 3410% (437 mg) of control weights by 150 days; total cell yields increased from 125% to 5890% (113.7×10^6 cells) of controls over the same treatment period. Chronic DES increased the proportion of PRL cells from 33% to 50% as revealed by immunocytochemistry. Intracellular PRL levels ranged from 1.95 to 9.40 times that of controls ($p<.05$). In addition, beyond 8 days of DES, PRL release in vitro from the experimental cell group was significantly ($p<.05$) and consistently greater than controls in 12 experiments. All these DES effects were more pronounced among ovariectomized rats. Some PRL cells in DES-treated rats had greater sedimentation velocities at unit gravity, suggesting increased cell size and/or density; moreover, 30% or more of the tumor cells (and none of the controls) sedimented through Ficoll-Hypaque ($1.08 g/cm^3$). Even though approximately equal proportions of PRL cells were recovered from the upper and lower Ficoll fractions, cells from the upper region released 3-4x more PRL in vitro. Bromocryptine treatment (4 mg/day/5days) in rats bearing 22-, 35-, 44- or 90-day tumors reduced pituitary weights, cell number, PRL content, and PRL release in vitro; the latter BC effect was more pronounced among the less dense PRL cells. Cell cycle analyses showed that the percentage of cells in $S+G_2$ ranged from 5 to 31% in ovariectomized controls and 28 to 52% in tumors. Bromocryptine treatment did not affect the $S+G_2$ population. Information obtained on 6 individual human prolactinoma cell suspensions (courtesy of M. Thorner and G. Tindall) consistently showed a high percentage of cells in G_2+S ($\bar{x}$=46%). In patients on BC therapy, this distribution was not altered. On the basis of these data we conclude that the DES-induced tumor offers a reasonable model for studying dopaminergic influence on cell growth and secretion in human prolactinomas. Supported by NIH grant CA 23248.

18.5 FAILURE OF PLASMA PROLACTIN LEVELS TO RISE AFTER A SECOND INJECTION OF SULPIRIDE. POST-RECEPTORIAL REFRACTORINESS? R. Collu and H. Cohen*. Neuroendocrine Research Laboratory, Pediatric Research Center, Hôpital Ste-Justine, Montreal, Quebec H3T 1C5, and INSERM Unité 34, Hôpital Debrousse, Lyon, France.

It is now well established that the secretion of prolactin (PRL) is under a tonic inhibitory control exerted through specific pituitary receptors by dopamine (DA). Some clinical as well as experimental data have indicated that previous exposure to neuroleptics may modify the PRL response to either antagonist or agonist dopaminergic drugs. This may indicate that treatment with neuroleptics has changed either the sensitivity of dopaminergic receptors or PRL metabolism. We have recently performed "in vivo" and "in vitro" studies in order to verify whether a previous single exposure to an antidopaminergic agent might modify pituitary DA receptors as well as the PRL response to a second challenge by the agent. For this purpose, adult male Sprague-Dawley rats, 250-300 g body weight, bearing a chronic indwelling catheter in the jugular vein were utilized. While a first injection of the antidopaminergic, substituted benzamide drug sulpiride 1 mg/kg induced a large rise of plasma PRL levels, a second injection given 2 h later was totally inactive although the pituitary content of the hormone was still 76% of the initial values. When the second injection was given 8 h after the first it was slightly effective, but when administered 24 h later it was as effective as the first. The second of two consecutive injections of haloperidol 5 mg/kg given at 2 h interval, or an injection of morphine 10 mg/kg given 2 h after sulpiride, were incapable of inducing a release of PRL. Two h after an injection of sulpiride 1 mg/kg, a 30 min period of immobilization stress as well as a second injection with larger doses (5, 10 or 20 mg/kg) of sulpiride induced a significant rise in plasma PRL levels. Apomorphine 0.1, 1.0 or 5.0 mg/kg was at least as effective an inhibitor of PRL secretion when given 2 h after sulpiride than when injected after saline. "In vitro" studies of dopaminergic binding sites with [^{3}H]Spiroperidol ([^{3}H]SPIR) revealed that in pituitary glands the apparent dissociation constant (K_D) and maximal number of binding sites (B_{max}) of [^{3}H]SPIR as well as the inhibitory constant (K_i) of two DA antagonists (haloperidol and sulpiride) and one DA agonist (bromocriptine) were not significantly modified by the drug. These data suggest that the only plausible explanation for the ineffectiveness of the second of two consecutive injections of sulpiride is the development of a state of refractoriness of the mechanisms that subserve the release of PRL induced by suppression of the inhibitory dopaminergic tonus.

18.6 OVARIAN STEROIDS MODULATE THE DENSITY OF ANTERIOR PITUITARY {^{3}H}-SPIPERONE BINDING SITES IN OVARIECTOMIZED RATS. Nancy S. Pilotte, David R. Burt and Charles A. Barraclough.* Depts. of Physiology and Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD. 21201.

Ovarian steroids affect the release of prolactin (PRL) into the peripheral circulation through 1) modulation of the release of hypothalamic dopamine (DA) into the hypophysial portal blood, 2) a direct stimulatory action on the anterior pituitary gland, and 3) desensitization of the anterior pituitary gland to the inhibitory action of DA. To clarify the mechanism(s) by which steroids affect the pituitary release of PRL, we tested whether the administration of estradiol (E_2) and/or progesterone (P) to ovariectomized (OVX) rats alters the number of available dopaminergic binding sites within the anterior pituitary gland.

All rats were OVX 7 days prior to the s.c. implantation of 2 30-mm Silastic capsules containing oil or 150 µg E_2 in oil. Forty-eight hr later, 2 30-mm Silastic capsules containing oil or 50 mg P in oil were implanted sc. At approximately 2, 6, 24 or 30 hr after the administration of P or oil, (48-76 hr after E_2), the anterior pituitary glands were removed, homogenized and stored at -20°C 1-4 weeks. The number of {^{3}H}-spiperone binding sites in the homogenate was assessed by incubation with 0.4 nM {^{3}H}-spiperone in the presence or absence of 10^{-6} M (+)-butaclamol for 15 min at 37°C. The incubation was terminated by addition of 3 ml of ice-cold 0.15 M NaCl. Bound radioactivity was separated from the free by rapid vacuum filtration through Whatman GF/B filters followed by 3 successive 3-ml rinses. The filters were equilibrated 12-16 hr in Liquifluor and counted.

No differences were observed in the specific binding of {^{3}H}-spiperone from the glands of OVX and OVX-E_2-treated rats removed 48-76 hr after E_2/oil treatment or from E_2P-treated rats removed 2, 6 or 30 hr after the administration of P (specific binding: 25.81 ± 4.48 (mean ± SE) to 33.66 ± 2.35 fmol/mg protein). However, the number of binding sites from the pituitary glands of E_2P-treated rats removed 24 hr after P was significantly increased ($p < .02$) to 43.50 ± 3.36 fmol/mg protein. In contrast, the peripheral levels of PRL are low in OVX rats, are moderately elevated in the mornings and rise sharply in a surge-like manner in the afternoons following E_2 or E_2P treatment. These findings suggest that, although there is a steroid-mediated change in the number of anterior pituitary receptors for DA, the availability of recognition sites for DA does not necessarily control the release of PRL. Other substances, perhaps stimulatory, may be more important in the regulation of PRL release under certain circumstances.

18.7 DISSOCIATION OF ESTROGEN RECEPTOR BINDING AFFINITY AND CONVERSION TO CATECHOL ESTROGENS: A PROBE FOR THE MECHANISM OF ACTION OF ESTROGENS IN THE CNS. D.G. Pfeiffer*, E. Barnea*, N.J. MacLusky*, L.C. Krey*, D.L. Loriaux*, and G.R. Merriam. Developmental Endocrinology Branch, NICHD, Bethesda, Md. 20205; Yale Univ., New Haven, Conn. 06510; and Rockefeller Univ., New York 10021.

Estrogens are metabolized in hypothalamus, pituitary, liver, and other tissues to 2- or 4-hydroxy catechol estrogens, which are able to interact with enzymes of catecholamine synthesis and degradation and possibly with membrane catecholamine receptors. It is not known whether this conversion plays an important role in mediating the physiological effects of estrogens in the CNS. To use as probes in the study of this problem, we sought to identify natural and synthetic estrogens which were impeded in conversion to catechol estrogens but had high estrogen receptor affinities, or which were readily converted to catechols but were poor receptor ligands. Of several estrogens tested, four--17α estradiol, 2- and 4-fluoroestradiol, and 11-β-methoxy-ethynyl estradiol (R2858) --appeared especially promising. Female Sprague Dawley rats, 200 g, were oophorectomized 5-7d before study. Cytosol estrogen (E) receptor affinities in hypothalamus-preoptic area-amygdala (HPA), pituitary, and uterus were studied at 4° and 25°C, using LH-20 columns to separate bound and unbound ligands. At 25°C, 17αE_2 had much lower E receptor affinity than the other estrogens.

IC-50's (pM) for displacement of [^{3}H]E_2 at 25°C (m±SE, n=3)

Compound	HPA	Pituitary	Uterus
17βE_2	0.39 ± 0.1	1.1 ± 0.2	1.2 ± 0.1
17αE_2	28.3 ± 4.3	64.6 ± 8.9	48.5 ± 6.4
4FE_2	0.9 ± 0.4	1.5 ± 0.3	1.5 ± 0.4
2FE_2	2.6 ± 0.8	10.6 ± 2.3	6.6 ± 0.8
R2858	4.0 ± 2.4	2.7 ± 0.8	3.6 ± 1.0

Conversion of these estrogens to catechol estrogens was studied in microsomes from HPA, pituitary, and liver, using a methylation system (catechol-O-methyltransferase, [^{3}H]S-adenosylmethionine) to convert newly synthesized catechol estrogens to stable [^{3}H]methyl ethers. [^{3}H]products were separated by HPLC. Conversion rates differed markedly. In liver, values were: 17βE_2: 40.6 pmol/min/mg microsome protein; 17αE_2: 176.5; 4FE_2: 31.6; 2FE_2: 11.5; R2858: 8.9. In HPA and pituitary, conversion rates were much lower; e.g., in HPA, rates were: 17βE_2: 1.08; 17αE_2: 1.48; other estrogens < 0.4. Thus 17αE_2 is readily converted to catechol estrogens but is a relatively poor E receptor ligand, while 2FE_2, 4FE_2, and R2858 are quite potent ligands but poorer substrates for catechol formation. If catechol estrogen formation is obligatory for expression of some CNS estrogen actions, one would expect 2FE_2, 4FE_2, and R2858 to be impeded in those actions, while 17αE_2 might retain potency despite reduced E receptor affinity.

18.8 CATECHOL ESTROGEN FORMATION IS NOT ESSENTIAL FOR ESTROGEN STIMULATION OF GONADOTROPHIN RELEASE AND FEMALE SEXUAL BEHAVIOR IN THE RAT. N.J. MacLusky*, L.C. Krey*, G.R. Merriam, B. Parsons* and F. Naftolin*, (SPON: A. Van den Pol). Yale University School of Medicine, New Haven, CT 06510; Developmental Endocrinology Branch, NICHD, Bethesda, MD 20205; and Rockefeller University, New York 10021.

Estrogens are extensively converted in the brain and pituitary gland to 2- and 4-hydroxylated "catechol" estrogens (CEs). The physiologic significance of these metabolic pathways remains uncertain. Since CEs can interact directly with pathways of catecholamine synthesis and metabolism, it is possible that local CE formation could participate, together with estrogen receptor mediated events, in estrogen actions on the CNS. One way of dissecting apart the contributions from estrogen receptor and catechol-mediated effects is to utilize structurally modified estrogens which have either a reduced receptor affinity [e.g. estradiol 17α (17αE_2)] or a reduced ability to act as substrates for catechol formation [e.g. 2- and 4-fluoroestradiol (2 and 4FE_2) and R2858] (Pfeiffer, et al, this meeting). We have used this approach to examine the role of CEs in the control of cyclic gonadotrophin release and feminine sexual behavior female Sprague-Dawley rats exhibiting regular 4-day estrous cycles were ovariectomized on the evening of diestrus 1. Twelve hours later (10am "diestrus 2") they were implanted subcutaneously with Alzet 2001 osmotic minipumps containing either 17βE_2, 17αE_2, 2FE_2, 4FE_2, R2858 or the infusion vehicle alone (propylene glycol; 0.1% ascorbic acid). The 17βE_2 infusion rate (0.025 ug/h) was chosen to reproduce the effects of the proestrus estrogen surge. Infusion rates for R2858, 2FE_2 and 4FE_2 were adjusted to give the same levels of cell nuclear estrogen receptors (ERn) in the brain and pituitary as those produced by the 17βE_2 infusion. 17αE_2 was infused at rates of 0.025 and 0.25 ug/h; neither of these 17αE_2 treatments significantly increased brain and pituitary ERn concentrations. Tail vein blood samples were withdrawn from all animals at 12 noon, 2, 4, and 6pm on the expected day of "proestrus" and assayed for LH. The animals were then injected s.c. with 1 mg progesterone in propylene glycol and tested for lordosis and proceptive behaviors 5 h later.

Treatment ug/h	N	LH at 6pm ng/ml±SEM	Lordosis Quotient % ± SEM	Proceptivity Frequency
Vehicle	7	77±18	0	0/7
17αE_2, 0.025	7	53±16	0	0/7
17αE_2, 0.25	7	44±9	0	0/7
17βE_2, 0.025	7	435±69	94±69	5/7
2FE_2, 0.1	7	645±221	84±9	6/7
4FE_2, 0.025	7	445±121	92±3	4/7
R2858, 0.05	7	475±98	87±2	6/7

All of the estrogens except 17αE_2 elicited LH surges and proceptive and lordosis behaviors. These results suggest that local CE formation is not an obligatory step in the mechanism by which estrogens induce LH release and female sexual behavior. (Supported by NIH grant HD13507).

18.9 REMOVAL OF THE NEUROINTERMEDIATE PITUITARY RESULTS IN AN EFFECTIVE INCREASE IN CORTICOID FEEDBACK. K. Fagin*, S. Wiener*, J. Shinsako* and M. Dallman* (SPON: M. Mangiapane). Dept. of Physiol., UC San Francisco, CA 94143 and Dept. Psychiatry, Stanford University, CA 94305.

We have reported that rats in which the neurointermediate pituitary had been removed (NX) have a selective deficit in their ability to increase plasma ACTH and corticosterone (B) following a noise ("neurogenic" or exteroceptive) stimulus compared with controls (NC). Plasma ACTH and B increases after 15 ml/kg hemorrhage ("systemic" or interoceptive) stimulus are similar in NX and NC rats (Endo Soc Abstr #838, 1982). We investigated here the cause of the selective deficit in ACTH and B secretion. Ability to perceive a neurogenic stimulus, indicated by latencies to pawlicking and jumping in a heat platform test were identical in NX and NC rats, but NX rats demonstrated the predicted deficits in plasma B elevations after the heat test as well as in a novel environment compared with NC rats ($p<0.05$). Since AM plasma B levels in NX rats are significantly higher than in NC rats, such levels might impose an increased negative feedback signal to ACTH secretion following neurogenic stimuli. We determined in cannulated rats that the elevated B levels did not indicate the lack of a diurnal rhythm; significant rhythms in plasma B and in water intake were present both in NX and in NC rats ($p<0.05$).

	ACTH (pg/ml)				B (µg/dL)			
(h)	1930	0230	0830	1430	1930	0230	0830	1430
NC	65±8	66±12	30±3	46±3	16.2±1.8	3.1±0.4	1.4±0.1	6.5±2.7
NX	59±6	63±17	56±15	58±9	15.1±1.6	5.4±1.1	11.2±1.5	14.3±2.0

(lights on 0830-2030 h. mean±SEM. NX n=5; NC n=4)

Adrenal sensitivity to ACTH appeared to decrease at 0230 h in both groups; however, B levels were elevated in NX rats by 0830 h, suggesting that by that time the normal diurnal rhythm in hormone levels was overridden by other factors. Since mean plasma B over 24 h was 1.7-fold higher in NX vs. NC rats ($p<0.05$), we thought that this chronic elevation in mean plasma B might attenuate the plasma ACTH increase after adrenalectomy, a low-intensity stimulus known to provoke a B-sensitive ACTH response. The increase in plasma ACTH 1 h after adrenalectomy was significantly less in NX rats compared with NC rats. Thus, NX rats have normal perception of and behavioral reactions to a neurogenic stimulus, a normal diurnal drinking rhythm, and capacities both for a rhythm in adrenal sensitivity to ACTH and to secrete large amounts of ACTH. The attenuated increase in ACTH after adrenalectomy indicates that the deficit in plasma B responses to neurogenic stimuli may result in part from a corticosterone negative feedback signal that is chronically elevated. Supported by AM28172 and MH15091.

18.10 CORTICOTROPIN RELEASING FACTOR ELICITS RESPONSES CHARACTERISTIC OF STRESS REACTION IN PRIMATES H.M. Schulte*, G.P. Chrousos*, P.W. Gold*, E.H. Oldfield*, G.B. Cutler* and D.L. Loriaux* (Spon: F.K. Goodwin) Developmental Endocrinology Branch, NICHD, Psychobiology Branch, NIMH, and Surgical Neurology Branch, NINCDS, NIH, Bethesda MD 20205

A 41 amino acid peptide possessing ACTH releasing activity has recently been isolated from ovine hypothalami. It has greater corticotropin (ACTH) releasing potency than any previously identified endogenous or synthetic peptide in pituicyte cultures in vitro and, in rats, subhuman primates and man in vivo. We report that in addition to its corticotropin releasing effects, corticotropin releasing factor (CRF) stimulates the secretion of growth hormone (GH) and prolactin (PRL) and influences the cardiovascular system as well. Intravenous CRF administered to pituitary stalk-sectioned cynomolgus macaques in graded doses (0.01 to 40 µg/kg, n= 26 stimulation tests) stimulated cortisol secretion with an ED_{50} of 0.5 µg/kg. It also released GH and PRL at all doses above 1 µg/kg. Plasma TSH and LH concentration were not altered. CRF (10 µg/kg iv bolus) also caused significant GH and prolactin release in rhesus monkeys with intact hypothalamic-pituitary units ($p < 0.05$). It did not influence TSH and LH secretion in these animals. In the same animals heart rate and blood pressure were measured at 1 minute intervals for 30 min before and for 2 h after CRF administration. CRF induced a marked transient increase in heart rate, with a peak at 5 min (193 + 7 vs 158 + 15, $p < 0.025$). Heart rate returned to control values by 50 min. No discernible changes in mean arterial blood pressure were noted. Thus, peripheral administration of CRF elicited several responses characteristic of the stress reaction in primates: cortisol secretion, GH and PRL release, and tachycardia. Work from other laboratories suggests that the increased heart rate results from a CRF-induced release of catecholamines. We conclude: 1. In the primate, CRF releases GH and PRL as well as ACTH; 2. The hormonal response to CRF is that usually associated with stress; 3. CRF causes an immediate and prominent increase in heart rate. These findings suggest that several aspects of the stress response may be mediated by CRF.

18.11 GNRH RELEASE FROM PERIFUSED MURINE HYPOTHALAMI, L. M. Johnson*. Divn. of Pediatric Endocrinology, U. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

A study of in vitro release of GnRH from murine hypothalmi is in progress. Such tissue provides less GnRH per animal than does rat tissue, but the much smaller tissue size facilitates diffusion of gasses to maintain cellular integrity. Brains of the mice were dissected on ice immediately after decapitation. Hypothalamic borders extended rostrally to include preoptic area, caudally to the mamillary bodies, laterally 1.0 mm from midline and dorsally 2 mm. Each hypothalamus was bisected through the midline and halves were placed in separate perifusion chambers. Each chamber held 2 halves. 0.5 ml fractions were collected over a 6 minute period into RIA tubes. Kelch anti-GnRH (10-15) was used. A sensitivity of 0.5 pg/tube was achieved in most assays using a procedure of overnight (4 C) incubation with unlabelled hormone plus anti-hormone, 3 hr (25 C) incubation with labelled hormone and rapid charcoal separation. The Kreb's buffer slightly depressed binding and was, thus, used in standards also.

Baseline release was measured over a 2 hr period for 0, 5 and 20 mM glucose. No release occurred without glucose. At 5 mM glucose, release varied, being only occasionally detectable in 2 intact mice but variable between 5 and 10 pg/fraction for 3 other intact, young adult male mice. For 2 hypothalami placed in 20 mM glucose, release was between 20 and 40 pg/fraction, with 4 pulses each in 2 hrs. Addition of 15 mM mannitol to 5 mM glucose did not enhance release.

Baseline release in young adult, ovariectomized mice varied from undetectable to 3 pg/fraction compared to release during 60 mM KCl stimulation that was 8 - 10 pg/fraction. Baseline release in 4 old (1.7 yr) mice (ovx at age 5 wk, as the others) was $\leq$2 - 2.7 pg/fraction (20 mM glucose) or $\leq$0.5 pg/fraction (5 mM glucose), but only one chamber (20 mM) contained slightly increased GnRH during potassium stimulation.

It appears that for murine hypothalami in vitro (1) 20 mM glucose supports higher baseline GnRH release than 5 mM glucose, (2) that release may be pulsatile under some conditions and (3) respond well to potassium stimulation in young but not in old ovariectomized mice.

18.12 IMMUNOCYTOCHEMICAL EVIDENCE FOR GONADOTROPIN RELEASING HORMONE NEURONAL AUTONOMY IN THE PRIMATE MEDIAL BASAL HYPOTHALAMUS. Paul C. Goldsmith, Lisa R. Brezina* and Yu-Chih Jao*. Dept. of Ob, Gyn and Repro. Sci., Univ. of California, San Francisco, CA 94143.

In order to determine the neuroanatomical basis for the reported gonadotropin releasing hormone (GnRH) autonomy of the deafferented primate medial basal hypothalamus (MBH), we studied the distribution and contacts between GnRH neurons within the intact MBH. Immunofluorescence and the PAP technique were performed on frontal vibratome sections from 5 male and 5 female neonatal baboons. The GnRH cell body (CB) and fiber distribution was identical for both sexes. At the optic chiasm, bilateral groups of about 20 CBs appeared dorsal to and partly within each supraoptic nucleus. More posterior CBs, along with those spilling over the medial border of each optic tract (OT) gave rise to a distinct fiber pathway extending to the infundibular lip (IL) which we have designated the ventral hypothalamic tract (VHT). In its lateral third, VHT CBs were dispersed and aligned within the tract. At the midpoint, CBs became scarce, but axons formed a loose bundle parallel to the ventral hypothalamic surface. More medially, VHT fibers condensed as they came to lie close to the ventral surface. Some vertically oriented bipolar CBs, perpendicular to the VHT, appeared amidst the granular cell layer at the ventral surface. Their axons ascended dorsally only to contact or join VHT fibers, while their ventral dendrites vanished into the pia. Occasional CBs within the VHT sent axons ventrally, or dendrites to contact nearby capillaries. At the IL, bilateral groups of about 10 GnRH CBs again appeared, primarily aligned within the VHT. These CBs were always lateral and/or ventral to the arcuate nucleus, but not within the nucleus itself. Compact VHT axons turned ventrally to enter the infundibulum (INF) from its perimeter. Beaded fibers gave rise to radiating collaterals, all of which terminated in a dense GnRH plexus in the palisade zone of the median eminence, covering the entire INF. Posterior to the INF, CBs were rare, but fibers accumulated near the small capillary network on the ventral surface.

The most interesting findings were GnRH intercellular contacts of diverse type and complexity. Close contacts between CBs occurred within the VHT, especially at the IL. Their proximity suggested electrotonic coupling rather than synaptic interaction. In addition, polygonal axo-axonic formations with terminal boutons were observed between 2 or 3 contributing, but more widely dispersed, CBs at the IL. Together, these contacts may recruit GnRH neurons into a phasic firing pattern and may help amplify pulsatile GnRH release. CBs within the MBH together with their prevalent contacts may be sufficient to ensure concerted circhoral GnRH secretion into portal blood, and to maintain basal gonadotropin levels in the primate, provided the VHTs and ILs are intact.

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18.13 ENDOGENOUS OPIATES DO NOT MEDIATE A SIMPLE TONIC INHIBITION OF GONADOTROPIN RELEASING HORMONE SECRETION IN THE MALE RAT. M. A. Miller*, D. K. Clifton*, D. M. Dorsa*, W. J. Bremner*, and R. A. Steiner* (SPONSOR: D. M. Bowden). Depts of Ob-Gyn, Psychology, Physiology and Biophysics, and Medicine, University of Washington and VA Medical Center, Seattle, WA 98195

Recent studies have shown that opiate antagonists enhance LH release and imply that endogenous opiate pathways tonically inhibit episodic gonadotropin releasing hormone (GnRH) secretion. We hypothesized that if this were true, then a continuous blockade of opiate receptors should result in a sustained increase in LH pulse frequency and/or amplitude. To test this hypothesis we examined the effect of continuous exposure to the opiate antagonist naloxone (NAL, Endo Labs) on LH pulse frequency and amplitude in the intact male rat.

Young adult male, Sprague-Dawley rats were implanted with jugular catheters under ether anesthesia. Beginning at 0900h on the next day, blood samples (400 µl) were taken every 10 min for 5 hours. After each sample, animals received an equal volume of a blood replacement mixture. Following sample #6, animals in the experimental group (n=8) received a bolus injection of 2 mg/kg NAL (IV) as well as an additional 0.2mg NAL every 5 minutes over the subsequent 4 hours. The control group (n=7) received saline in place of naloxone. Plasma LH levels were measured with NIAMDD rat LH RIA reagents.

Three of 7 control animals exhibited spontaneous LH pulses at apparently random intervals during the injection period. NAL effected an immediate (within 20 min) pulsatile release of LH (from preinjection levels of 9±2 to 42±5 ng/ml) in 7 of 8 animals, whereas none of the control animals exhibited an LH pulse for at least 30 minutes following saline injection; the two groups were significantly different by this 30 minute cut-off criterion ($p<0.01$). This stimulated LH release was not sustained during chronic opiate blockade. Average serum LH levels during the last 2 hours of NAL treatment ($\bar{X}$=7±1 ng/ml) did not differ significantly from pretreatment levels. Although animals receiving the NAL treatment exhibited a greater number of LH pulses during the treatment period, the difference in frequency between the groups was not significant (control: 0.32±0.18 vs experimental: 0.66±0.10 pulses/hour, $p>0.1$).

Conclusion: Although endogenous opiates may interact with the GnRH system, these opiate pathways do not exert a simple tonic inhibition of pulsatile GnRH output.

18.14 PLASMA β-ENDORPHIN (βEND), β-LIPOTROPIN (βLPH) AND ACTH RESPONSES TO STRESS IN INTACT AND ADRENALECTOMIZED RATS. Errol B. De Souza and Glen R. Van Loon. Departments of Physiology and Medicine, University of Toronto, Toronto, Ontario, Canada and Department of Medicine, University of Kentucky and Veterans Administration Medical Center, Lexington, KY.

It has been proposed that βEND, βLPH and ACTH are concomitantly secreted from the pituitary gland in an equimolar ratio in response to a variety of stimuli including stress and adrenalectomy. The parallel secretion of these peptides probably relates to their common origin and biosynthesis from a larger precursor glycoprotein referred to as proopiomelanocortin. The present study was designed to compare the plasma concentrations of βEND, βLPH and ACTH under basal conditions and following application of a 2 min restraint stress both in intact and chronically adrenalectomized adult male rats. In intact rats, application of a 2 min restraint stress produces rapid, parallel increases in plasma concentrations of radioimmunoassayable βEND/βLPH and ACTH, peaking at 2.5 to 5 min following onset of the stress and returning almost to basal concentrations by 30 min. The antibody used in the βEND/βLPH radioimmunoassay detects βEND and βLPH equally on a molar basis. In order to determine the relative amounts of βEND and βLPH that are present in plasma of control, nonstressed rats and the amounts that are secreted in response to stress, we separated the peptides by gel exclusion chromatography prior to assay of the peptides by radioimmunoassay. Plasma obtained from control, nonstressed rats contains more βEND than βLPH. Equal amounts of βEND and βLPH are present in plasma of intact rats 5 min after the onset of a 2 min restraint stress as assessed by gel exclusion chromatography and radioimmunoassay. Rats adrenalectomized nine days earlier have significantly higher basal plasma concentrations of βEND/βLPH and ACTH than are present in intact rats. Furthermore, the plasma responses of both βEND/βLPH and ACTH to stress are markedly enhanced in adrenalectomized rats when compared to the corresponding responses in intact rats. Gel exclusion chromatography revealed that both the higher basal concentration and the enhanced plasma βEND/βLPH response to stress in adrenalectomized rats result primarily from increases in the βLPH component with lesser increases in the βEND component. In summary, the data of the present study demonstrate that following application of a 2 min restraint stress, equal amounts of βEND and βLPH are secreted in intact rats whereas, greater amounts of βLPH than βEND are secreted in chronically adrenalectomized rats. Thus, different mechanisms appear to regulate the βEND and βLPH responses to stress in the normal state and following removal of glucocorticoid negative feedback.

18.15 PROLACTIN INCREASES THE ACTIVITY OF TUBEROINFUNDIBULAR AND NIGRONEOSTRIATAL DOPAMINE NEURONS: PROLACTIN ANTISERUM INHIBITS THE HALOPERIDOL-INDUCED INCREASES IN DOPAMINE SYNTHESIS RATES IN MEDIAN EMINENCE AND STRIATUM OF ADULT MALE RATS. Glen R. Van Loon, Andrew Shum*, Susan R. George* and Seon Shin*. Departments of Medicine, University of Toronto, Toronto, Canada, and University of Kentucky and Veterans Admin. Medical Center, Lexington KY, and Department of Physiology, Queen's University, Kingston, Canada.

Important differences in the regulation of tuberoinfundibular and nigroneostriatal dopaminergic neurons have been proposed. For example, neuroleptics such as haloperidol increase striatal dopamine synthesis and release promptly through a dopamine-receptor mediated negative feedback mechanism. In contrast, haloperidol increases median eminence dopamine turnover only after a prolonged latent period; it has been postulated that this haloperidol effect is mediated by prolactin since it is not present in hypophysectomized animals. The present study was designed to provide conclusive evidence for this thesis, and to assess further a possible effect of prolactin to alter striatal dopamine turnover. We examined the effect of prolactin antiserum on the haloperidol-induced increases in median eminence and striatal dopamine synthesis rates in adult male rats. Our prolactin antiserum generated in rabbits effectively precipitated prolactin, but not other anterior pituitary hormones. Following iv administration, a large excess of circulating prolactin antibody could be demonstrated, even 24 hours after haloperidol administration. Groups of rats received haloperidol 2.5 mg/kg or tartaric acid vehicle sc twice, 22 h and 12 h before measurement of brain dopamine turnover. Comparable groups of haloperidol or vehicle-treated rats received either prolactin antiserum iv or normal rabbit serum. Dopamine synthesis turnover rate was estimated by measurement of accumulation of L-dihydroxyphenylalanine following inhibition of L-aromatic amino acid decarboxylase with m-hydroxybenzylhydrazine. Haloperidol increased median eminence dopamine synthesis rate, and prolactin antiserum completely prevented this effect. Prolactin antiserum did not alter basal median eminence dopamine synthesis rate in male rats. In addition to its effect in median eminence, prolactin antiserum blunted the haloperidol-induced increase in striatal dopamine synthesis rate, suggesting that the haloperidol-induced increase in nigroneostriatal dopamine turnover is mediated in part by prolactin. Neither haloperidol nor prolactin antiserum altered serotonin synthesis rate in mediobasal hypothalamus or striatum. The data provide further evidence that the haloperidol-induced increase in activity of tuberoinfundibular dopamine neurons is mediated by prolactin, and further support for a mechanism by which prolactin can regulate its own secretion.

18.16 ANGIOTENSIN II RECEPTORS IN BRAIN POTENTIATE THE STIMULATORY EFFECT OF ENDOGENOUS OPIOID NEURONS ON CENTRAL SYMPATHETIC OUTFLOW. Nathan M. Appel and Glen R. Van Loon. Departments of Physiology and Medicine, University of Toronto, Toronto, Ontario, Canada and Department of Medicine, University of Kentucky and Veterans Administration Medical Center, Lexington, KY.

Endorphins, endogenous opioid peptides, appear to have neurotransmitter or neuromodulator function in brain, mediating a wide variety of effects. We have reported that intracisternal administration of synthetic human β-endorphin increases plasma concentration of catecholamines, presumably by acting at unknown brain site(s) to increase sympathetic outflow to the adrenal medulla and sympathetic nerves. The β-endorphin-induced increase in plasma catecholamines was prevented by ganglionic blockade with chlorisondamine and by bilateral adrenal denervation. Subsequently, we provided evidence for the involvement of central cholinergic, noradrenergic and somatostatin neurons in the mediation of this endorphin-induced stimulation of sympathetic outflow. A variety of other neurotransmitters may be involved. In the present study we examined the possibility that angiotensin II, acting in brain, modulates endorphin-induced catecholamine secretion. Synthetic human β-endorphin, 1.45 nmoles administered intracisternally, increased plasma epinephrine and norepinephrine concentrations 10-30 minutes later when compared to the response in animals receiving saline vehicle. Simultaneous administration of angiotensin II, 1.0 nmole intracisternally together with β-endorphin, potentiated the plasma epinephrine and norepinephrine responses to intracisternal β-endorphin. In contrast, simultaneous administration of the angiotensin II receptor antagonist [Sar^1,Val^5,Ala^8]-angiotensin II (saralasin), 1.1 nmoles intracisternally together with β-endorphin, blunted the plasma epinephrine and norepinephrine responses to β-endorphin. These data are consistant with the hypothesis that activation of angiotensin II receptors in brain potentiates the endorphin-induced stimulation of central sympathetic outflow. It remains to be demonstrated whether angiotensin II acting in brain to modulate activity of opioid neurons is synthesized in brain or is derived peripherally.

18.17 RELEASE OF VASOPRESSIN AND β-ENDORPHIN INDUCED BY ANGIOTENSIN IN THE CONSCIOUS RAT: STUDIES ON THE MECHANISM OF ACTION.
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Intravenous infusions of angiotensin I and II induce release of β-endorphin from the anterior lobe and of vasopressin from the posterior lobe of the pituitary gland. Captopril, a converting enzyme inhibitor, abolishes the effect of angiotensin I, whereas saralasin blocks the action of angiotensin II.

(i) A fornix lesion anterior and ventral to the subfornical organ inhibits the effect of angiotensin II on vasopressin release, but does not effect the release of β-endorphin. (ii) In rats with hereditary lack of vasopressin the angiotensin II-induced release of β-endorphin was diminished as compared to controls. (iii) When rat medial basal hypothalami were incubated in vitro angiotensin II increased the release of β-endorphin.

We conclude that blood-borne angiotensin II releases the pituitary hormones via different routes: vasopressin release from the posterior lobe may be mediated by the subfornical organ; in contrast, β-endorphin release from the anterior lobe may be due to a direct effect of angiotensin on the adenohypophysis as well as to an enhanced release of vasopressin from the external layer of the median eminence which then acts as a β-endorphin releasing factor.

18.18 HISTAMINE LEVELS ARE SELECTIVELY DECREASED IN THE POSTERIOR PITUITARY LOBE OF BRATTLEBORO RATS LACKING VASOPRESSIN.
H.H. Holcomb§, P.M. Gross§§, M. Kadekaro§§, and J.M. Saavedra§§§. Biological Psychiatry Branch§, Laboratory of Cerebral Metabolism§§, and Laboratory of Clinical Science§§§, National Institute of Mental Health, Bethesda, Maryland 20205.

Histamine may have an important role in modulating the secretion of vasopressin in normal animals. Its levels in the posterior pituitary lobe are high (Saavedra, J.M., et al., J. Neurochem., 25:257, 1975), and intracerebroventricular administration of histamine causes a marked increase in circulating levels of vasopressin (Dogterom, J., et al., Experientia 32:659, 1976; Tuomisto, L., et al., Eur. J. Pharmacol., 63:15, 1980).

Our demonstration of high histamine methyl transferase activity in the posterior pituitary lobe of normal rats (apparent activity: 50±7.9 pmoles/μg protein/h; mean±SE, n=6) suggests a rapid rate of histamine turnover. In marked contrast, the intermediate lobe exhibits no detectable histamine methyl transferase activity (n=6) despite high histamine content.

We studied histamine metabolism in homozygous (DI) and heterozygous (HZ) Brattleboro rats deficient in vasopressin. We demonstrate a marked reduction in posterior pituitary histamine content of DI rats in comparison with HZ controls. Conversely, histamine levels were not different in the intermediate pituitary lobe of DI and HZ rats.

	Histamine Levels (ng/mg protein)	
	DI	HZ
Intermediate Lobe (n=4)	3.3 ± 1.0	3.5 ± 0.27
Posterior lobe (n=9)	4.9 ± 0.65	14.8 ± 2.72*

* $p < 0.001$

Our results suggest that these marked differences in histamine content in the posterior pituitary lobe may reflect its role in modulating vasopressin secretion and water balance.

18.19 SELECTIVE INCREASE IN THE RATE OF GLUCOSE UTILIZATION BY THE NEUROHYPOPHYSIS DURING DEHYDRATION FROM SALINE INGESTION.
P.M. Gross, M. Kadekaro, H.H. Holcomb§, L. Sokoloff, J.M. Saavedra§§. Laboratory of Cerebral Metabolism, Biological Psychiatry Branch§, and Laboratory of Clinical Science§§, National Institute of Mental Health, Bethesda, Maryland 20205.

Dehydration is associated with increased synthesis and elaboration of vasopressin from the hypothalamo-neurohypophysial tract. Previous studies in this laboratory with the 2-[^{14}C]deoxyglucose method used qualitatively provided autoradiographic evidence that chronic saline ingestion activated metabolism in the neurohypophysis but not in the hypothalamic nuclei of origin of this tract (Schwartz, W.J., et al., Science, 205:723-725, 1979). In the present studies we have essentially repeated those earlier studies but with the quantitative 2-[^{14}C]deoxyglucose method to assess the magnitude of the changes in glucose utilization. Male Sprague-Dawley rats (318-371 g initial weight) were provided water or 2% saline (n=5 in each group) ad libitum over a 5 day period. Animals which drank saline had an average 18% loss in body weight and an increase in plasma osmolality of 20 mosm/l over controls. Values for glucose utilization in selected structures of conscious animals were (means ± SE in μmoles/100 g/min, * $p < 0.05$):

Structures	Water	Saline	S-W % Diff
N. paraventricularis	63 ± 3	62 ± 3	-2
N. supraopticus	71 ± 4	72 ± 4	+1
N. suprachiasmaticus	71 ± 7	70 ± 6	-1
Area retrochiasmaticus	58 ± 4	52 ± 4	-7
Neurohypophysis	45 ± 9	102 ±10*	+127

The results confirm that dehydration from chronic saline ingestion causes a dramatic increase in metabolic activity in the neurohypophysis but not in hypothalamic nuclei that synthesize or store vasopressin.

18.20 MOTILIN STIMULATES GROWTH HORMONE RELEASE IN VITRO AND IN VIVO.
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The peptide motilin has been localized to the anterior pituitary gland and hypothalamus-median eminence, as well as the gut, by immunohistochemistry and radioimmunoassay (Jacobowitz et al., O'Donohue et al., respectively, Peptides 2; 1981). These findings suggested a role for motilin in anterior pituitary function. In a previous report by us (Samson et al., Brain Research Bulletin 8; 1982), a single dose (10^{-6}M) of motilin released growth hormone (GH) from rat hemipituitaries and dispersed anterior pituitary cells. This result led us to seek here a dose-related ability of motilin to release GH from dispersed anterior pituitary cells and, as well, an action to release GH in vivo. In 2 experiments, anterior pituitaries from adult male rats were dispersed and cultured overnight. The dispersed cells were incubated with synthetic motilin (Peninsula) at log and half-log doses ranging from 5×10^{-8}M to 10^{-5}M. The minimal effective dose of motilin which caused a significant release of GH was 10^{-7}M. Increasing doses of motilin produced a linear, dose-related increase in GH release ($p<.02$ to $p<.001$). By contrast, TRH in the same system only stimulated GH release slightly at 10^{-6} or 5×10^{-6}M doses. Motilin also was incubated with dispersed anterior pituitary cells from ovariectomized estrogen-progesterone-primed rats. Again motilin increased the secretion of GH into medium at doses of 10^{-6} ($p<.001$) and 10^{-5}M ($p<.01$) in a dose-related fashion. In vivo, the intravenous (iv) injection of 1 or 10 μg of motilin into conscious adult male rats bearing jugular cannulae produced variable results, while 100 μg motilin iv significantly elevated plasma GH 2-fold 5 min post-injection.

Finally, microinjection of motilin (5 μg) into the 3rd ventricle of conscious adult male rats paradoxically lowered plasma GH 5 min post-injection ($p<.05$) when compared to preinjection values. This paradoxical effect may indicate that an ultrashort-loop feedback mechanism exists whereby exogenous motilin may decrease hypothalamic motilin secretion, thereby reducing the motilin stimulus for GH release. The specificity of the motilin effect on GH was attested to by the failure of motilin to alter significantly PRL, TSH and LH in each paradigm. We conclude that motilin may act physiologically on cells of the anterior pituitary at various doses to release GH specifically and in a dose-related fashion and that it might be involved in ultrashort-loop feedback to reduce its GH-releasing action. (Supported by NIH Grants HD-07062, HD-09988, and AM-10073).

18.21 MULTIPLE OPIATE RECEPTOR REGULATION OF PROLACTIN AND GROWTH HORMONE SECRETION. J.I. Koenig*, M.A. Mayfield* and L. Krulich* (SPON: J.G. Parnavelas). Dept. of Physiol., Univ of Texas Hlth. Sci. Ctr., Dallas, TX 75235.

Opioid peptides, as well as morphine have been shown to stimulate the secretion of both prolactin (PRL) and growth hormone (GH). However, receptor binding studies indicate that these compounds exhibit different affinities for the various opiate receptors. The present study was undertaken to determine whether specific opiate receptor subtypes mediate opiate-induced PRL and GH secretion. Adult male Sprague-Dawley rats (300-350 gm) were used in all the following experiments. Third cerebral ventricle (IVT) injections were performed through cannulae which had been implanted one week previously. Blood samples were obtained from chronic jugular cannulae which were implanted 48 hrs. before experimental use. Morphiceptin (MPC) was used as a rather selective ligand of the mu receptors, while delta receptor peptide (DRP) and [D-Ala^2, D-Leu^5] enkephalin (DADLE) were used as delta receptor agonists, respectively and bremazocine was used to stimulate the kappa receptors. The IVT injection of MPC, DRP and DADLE all dose dependently increased plasma PRL 15 min following injection over a dose range of 100 ng to 10 µg. These effects were all significantly reversed by naloxone (0.6 mg/kg, iv) given 15 min before the IVT injection. Bremazocine also elevated PRL in a dose dependent fashion over a range of 0.0125 to 0.5 mg/kg, i.v. The PRL-releasing effect of 0.125 mg/kg of bremazocine was not reversed by 0.6 or 6.0 mg/kg of naloxone or MR 2266, a specific kappa receptor antagonist. The opiate influence on GH secretion exhibited different characterisitics. Bremazocine regardless of the dose had no effect on GH secretion. DRP stimulated GH over the dose range of 100 ng to 10 µg and except for the 100 ng dose the effect was not attenuated by naloxone (0.6 mg/kg). DADLE stimulated GH at a dose of 500 ng which was not blocked by naloxone, similar to DRP. These results indicate that the secretion of PRL may result from activation of mu or delta receptors because naloxone inhibited the effect of MPC but also the effect of DADLE or DRP, which is a rather specific delta agonist. The PRL releasing effect of bremazocine, not blockable by naloxone implies an entirely different mechanism. GH secretion seems to be controlled predominantly by the delta receptors, since naloxone is ineffective against DADLE or DRP and since MPC is effective only at high dose which may also activate the delta receptor. Kappa receptors are probably not involved either since bremazocine did not stimulate GH secretion. Supported by NIH Grant #HD 09988.

18.22 A POSSIBLE ROLE FOR OXYTOCIN IN THE CONTROL OF PROLACTIN RELEASE. W.K. Samson*, M.D. Lumpkin*, G.P. Kozlowski and S.M. McCann. Dept. of Physiology, UTHSCD, Dallas, Texas 75235.

Recently, we have reported the potent stimulatory action of oxytocin (OXY) on the in vitro release of PRL from rat hemipituitaries and dispersed cell preparations (Lumpkin et al., Endocrine Society, 1982). In order to further elucidate the possible role of OXY in PRL release, attempts were made to 1) correlate fluctuations in serum levels of both hormones after estrogen priming, 2) examine a possible depletion of neural lobe OXY during steroid-induced PRL secretion and 3) examine the effect of passive immunoneutralization of endogenous OXY on basal PRL secretion. Antiserum raised in rabbits to synthetic OXY (GPK936) was used for RIA purposes and generally binds 30% of total at a final dilution of 1: 25,000. No cross-reactivity was demonstrated to twenty other known hypothalamic and/or pituitary peptides in doses as high as 1 ug/tube, except in the case of vasopresin (AVP) where 1 ug AVP cross-reacted with the equivalency of 28 pg of OXY. Ovariectomized (OVX) rats were injected s.c. with 5 µg estradiol benzoate (EB) and sacrificed at 10 AM, noon, 1 and 2 PM two days later. A significant rise in serum PRL levels was observed following the 10 AM sampling time (10.26 ± 4.95 ngm PRL/ml) with highest values being detected at 1 pm (33.07 ± 8.82). A similarly significant rise in serum OXY with levels peaking at 1 pm (39.15 ± 1.5 pg OXY/ml) was seen. Serum OXY levels also were elevated significantly when compared to the 10 AM sampling (33.36 ± 1.02) at noon (37.90 ± 1.33) and 2 PM (38.70 ± 1.02).
In a second experiment OVX rats were decapitated hourly from 10 AM to 2 PM, 48 hrs. after EB injection. Serum PRL levels again were elevated significantly at 1 PM (33.13 ± 1.63) over those at 10 AM (20.2 ± 3.83). Neural lobe OXY content (ngm OXY/mg tissue) declined in a linear fashion from 11 AM (139.03 ± 13.34) to lowest values at 2 PM (81.70 ± 6.68, $p<.01$). Anterior lobe OXY content increased after 10 AM (2.82 ± 0.54 pg/ng tissue) with significantly higher values present at 1 pm (5.31 ± 0.77). Finally, antiserum to OXY (100 µl) was injected into freely moving, intact male rats through an implanted jugular cannula. Plasma PRL levels did not differ significantly in normal rabbit serum or anti-OXY treated rats at any sampling time (0-120 minutes). We have demonstrated previously the potent in vivo and in vitro PRL-releasing activity of OXY. Additionally, we have observed immunostaining for OXY in the external layer of the anterior median eminence, suggesting delivery of the peptide into the portal capillaries. Our data suggests a temporal correlation between OXY release from neuronal elements and steroid-induced PRL secretion. A role for OXY in basal PRL release seems unlikely however due to the failure of immunoneutralization of endogenous serum OXY levels to alter PRL secretion.

18.23 THE EFFECTS OF MORPHINE INJECTIONS DURING PREGNANCY IN THE RAT ON PLASMA LEVELS OF TSH AND PROLACTIN AFTER BIRTH. W.J. Litto*, J.P. Griffin*, and J. Rabii, Department of Biological Sciences and the Bureau of Biological Research, Rutgers University, Piscataway, New Jersey 08854.

The administration of opiates has been shown to lead to acute alterations in pituitary hormone secretion. Relatively little attention has been given to the possible long-term effects of opiate exposure on neuroendocrine systems. We have studied the influence of morphine sulfate (MS) injections, on days 5-12 of pregnancy, on TSH secretion in the offspring and on prolactin (PRL) release in the lactating dam. Pups born to MS or saline treated dams were sacrificed by decapitation at 5-day intervals between 5 and 35 days of age. Both male and female offspring of either MS or saline treated mothers exhibited a peak in their plasma TSH concentrations at 20 days of age. Plasma TSH levels before 20 days of age tended to be lower in the MS exposed pups as compared to the saline controls. TSH levels after day 20, on the other hand, were generally higher in MS treated animals. When the acute response of TSH to MS injection was studied in pups born to MS or saline treated dams, marked decreases in TSH release were seen in both sexes of either treatment group. No significant differences were apparent between the prenatally MS and saline exposed pups in their response to a single postnatal injection of MS. Pups were separated from their mothers between days 5 and 10 postpartum. Blood samples were obtained from the dam immediately before and at times after the return of the pups to the cage. Pups were returned after a 6-hour separation. The MS and the saline injected dams were both able to exhibit a significant PRL release in response to the suckling stimulus. The response of the MS group, however, was markedly lower than that of the saline controls. These experiments suggest that, at least in the 5 to 35 day old pups, there are apparent influences of prenatal exposure to MS on basal TSH release but not on the MS-induced acute inhibition of TSH secretion. Furthermore, MS injections during pregnancy appear to be capable of lowering the magnitude of the PRL secretion in response to suckling. Supported by National Institute of Drug Abuse Grant DA02227.

18.24 ADENOHYPOPHYSEAL DOPAMINE CONTENT DURING PHYSIOLOGIC AND PHARMACOLOGIC-INDUCED CHANGES IN PROLACTIN SECRETION. **K.T. Demarest, G.D. Riegle*, and K.E. Moore. Depts. of Pharmacol./Toxicol. and Physiol., Michigan State Univ., East Lansing, MI 48824.**

Dopamine (DA) released from terminals of tuberoinfundibular neurons is transported via hypophyseal portal blood to the anterior pituitary gland (AP). Here, the DA is either bound to receptors on cell membranes and/or is internalized and bound to prolactin containing granules within the lactotroph (Endocrinology 107: 30, 1980). It has been suggested that incorporation of DA into the AP is functionally related to the regulation of prolactin secretion. Studies were undertaken to characterize the relationship between AP DA and changes in prolactin secretion which occur in selected physiologic states and following drug treatments. Surges of prolactin secretion which occur on the afternoon of proestrous, during suckling, early pregnancy and restraint stress are all accompanied by a decrease in AP DA content. On the other hand, the increases in serum prolactin concentrations which are observed in aged rats or following water deprivation are accompanied by a dramatic increase in AP DA content. These results demonstrate that there is not a simple inverse relationship between AP DA and alterations in prolactin secretion.

An analysis of pharmacologic manipulations which alter prolactin secretion support this contention. DA agonists (apomorphine and bromocriptine) which decrease serum PRL do not alter AP DA. d-Amphetamine and L-DOPA, drugs which increase DA in the hypophyseal portal blood and decrease serum PRL, increase AP DA. Haloperidol and estradiol both increase serum PRL and decrease AP DA. α-Methyltyrosine (αMT), γ-butyrolactone (GBL) and reserpine, drugs which increase serum PRL by decreasing DA in the hypophyseal portal blood by interfering with the synthesis, release or storage of DA in tuberoinfundibular neurons, all decrease AP DA. The αMT- and GBL-induced increase in serum PRL and decrease in AP DA are blocked by pretreatment with bromocriptine or L-DOPA. The reserpine-induced increase in serum PRL but not the reduction of AP DA are blocked by bromocriptine or L-DOPA, suggesting that reserpine has a direct effect on the storage of DA in the AP. In addition, these data suggest that the ability of DA to inhibit PRL secretion is independent of the incorporation of this amine into the AP. (Supported by USPHS grant AG2644 and a MSU College of Osteopathic Medicine Biomedical Research Grant.)

18.25 ROLE OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS IN THE DIURNAL-NOCTURNAL SURGES OF PROLACTIN SECRETION DURING EARLY PREGNANCY. G.D. Riegle*, K.E. Moore and K.T. Demarest (SPON: J.L. Bennett). Depts. of Pharmacol./Toxicol. and Physiol., Michigan State Univ., East Lansing, MI 48824.

A diurnal nocturnal pattern of tuberoinfundibular dopamine (TIDA) nerve activity occurs out of phase with the diurnal-nocturnal surges of prolactin secretion during early pregnancy (Neuroendocrinology 34: 229, 1982). Since increases in serum prolactin concentrations stimulate TIDA neuronal activity (Frontiers in Neuroendocrinology 7: 161, 1982) the role of prolactin feedback in initiating and timing the biphasic patterns of TIDA nerve activity and prolactin secretion was considered. Studies were undertaken to determine the effect of manipulating the surges of prolactin on the biphasic changes in TIDA nerve activity during day 6 of pregnancy. TIDA nerve activity was estimated by measuring the in vivo rate of DA synthesis in the median eminence (the rate of DOPA accumulation 30 min after a decarboxylase inhibitor, NSD 1015, 100 mg/kg, ip). In vehicle-treated pregnant rats a diurnal-nocturnal pattern of prolactin secretion was observed with peaks at 600 and 2100 h and nadir at 1200 h. A biphasic pattern of TIDA nerve activity was also observed with a peak at 1200 h and nadirs at 300-600 h and 1800-2100 h. Pretreatment with haloperidol (2.5 mg/kg, s.c.) 24 h prior to sacrifice maintained high serum prolactin concentrations (700-800 ng/ml) throughout the day; DOPA accumulation in the median eminence was also increased, and the diurnal-nocturnal pattern was still maintained. Similarly, intracerebroventricular infusion of prolactin (1 µg) 12 h prior to sacrifice increased DOPA accumulation in the median eminence and the diurnal-nocturnal pattern was still observed. In these animals, the magnitude of the surges of prolactin secretion were reduced, probably reflecting increased TIDA neuronal activity. Pretreatment with bromocriptine (3 mg/kg, s.c.) 24 and 12 h prior to sacrifice reduced serum prolactin concentrations (<25 µg/µl) throughout the day; DOPA accumulation in the median eminence was also reduced but the diurnal-nocturnal pattern was lost. These results suggest that the prolactin-induced activation of TIDA neurons is not the cause of the daily changes in the activity of these neurons. Prolactin-induced activation of these neurons does, however, appear to be required during pregnancy for maintenance of "basal" TIDA nerve activity. (Supported by USPHS grants NS09174 and AG2644.)

18.26 PLACENTAL LACTOGEN MIMICS PROLACTIN IN ACTIVATING TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS. N.J. Duda*, K.T. Demarest, G.D. Riegle* and K.E. Moore (SPON: T.M. Brody). Depts. of Pharmacology/Toxicology and Physiology, Michigan State Univ., East Lansing, MI 48824.

During the first half of pregnancy in rats tuberoinfundibular dopamine (DA) neurons exhibit a daily biphasic pattern of activity which appears to be temporally related but out of phase with the diurnal-nocturnal surges of prolactin (Neuroendocrinology 34: 229, 1982). By day 13 of pregnancy the surges of prolactin cease and tuberoinfundibular neuronal activity is continuously maintained at an elevated level; these could be mediated by the increased secretion of placental lactogen which occurs at midpregnancy. The present experiments were undertaken to determine if placental lactogen can stimulate tuberoinfundibular DA neurons. The activity of these neurons was estimated from the in vivo rate of DOPA accumulation in the median eminence after the administration of 3-hydroxybenzylhydrazine (100 mg/kg, i.p.). An initial study was undertaken to determine if a factor in the serum of pregnant rats could stimulate tuberoinfundibular DA neurons. Female Long-Evans rats, ovariectomized for two weeks, were administered pregnant rat sera (collected on day 13-16 from pregnant rats) 3 ml, i.p. at 24, 18, 12 and 6 h prior to sacrifice. For comparison rat prolactin (RP-2-B, 10 µg/10 µl) was administered by intracerebroventricular (icv) infusion 12 h prior to sacrifice via previously implanted cannula guides. The pregnant rat sera, like the icv prolactin infusion, significantly increased the rate of DOPA accumulation in the median eminence. Thus, some factor in pregnant rat sera, perhaps placental lactogen, is capable of activating tuberoinfundibular DA neurons. Studies were undertaken to characterize the action of placental lactogen on tuberoinfundibular DA neuronal activity using human placental lactogen (HPL) since purified rat placental lactogen was unavailable. Tuberoinfundibular DA neurons were activated with 10-100 µg HPL when measured 12 h after icv infusion, while no effect was seen after 3 µg. Following icv infusions of HPL (10 µg) the rate of DOPA accumulation in the median eminence was accelerated at 12 and 16 h, but was without effect at earlier times (4 and 8 h). Thus, HPL exhibits a delayed onset of action similar to that seen with icv prolactin (Brain Res. 195: 236, 1980). These data indicate that placental lactogen can stimulate tuberoinfundibular DA neurons and this process could play a role in terminating the surges of prolactin at midpregnancy. (Supported by USPHS grant NS09174 and a College of Osteopathic Medicine Biomedical Research Grant.)

18.27 CALCIUM IONOPHORE A-23187 STIMULATES CYCLIC AMP AND PROLACTIN RELEASE IN ANTERIOR PITUITARY CELLS: ANTAGONISM BY DOPAMINE OR PENFLURIDOL. G. Schettini,* R.M. MacLeod* and M.J. Cronin (SPON: R.J. Krieg). Depts. of Medicine and Physiology, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

In many systems a relationship exists between Ca^{2+} and cyclic AMP (cAMP) to regulate cellular events. Both messengers are involved in the control of prolactin (PRL) release, although the association between Ca^{2+} and cAMP at the pituitary level is not clear. Dopamine (DA) inhibits PRL secretion, but the post-receptor mechanism of action of DA is poorly understood. We investigated the effect of Ca^{2+} ionophore A-23187 (CaI) on cAMP cellular content and PRL release in primary cultures of anterior pituitary cells from male rats. The effects of DA or penfluridol (PF), a neuroleptic that inhibits calmodulin biological activity, on basal or CaI-stimulated cAMP accumulation and PRL release were examined. cAMP (pmole/well) and PRL (ng/well) were measured by RIA. The cells were pretreated with isobutylmethylxantine (0.2 mM) for 2 h before the experiments, which lasted 15 min. CaI (1,5,10 µM) stimulated cAMP accumulation (control 3.6 ± 0.4; CaI 1 µM 5.8 ± 0.8, $p<0.05$; CaI 5 µM 10.0 ± 1.1, $p<0.001$; CaI 10 µM 9.9 ± 1.4, $p<0.01$) and PRL release. DA (0.5, 1, 5, 10 µM) and PF (0.5, 1, 10 µM) both inhibited basal cAMP accumulation and PRL release ($p<0.05$ to $p<0.01$). The table presents the results of another experiment in which the cells were simultaneously exposed to DA or PF with or without CaI. Both compounds show a clear ability to reduce both basal and CaI-stimulated accumulation of cAMP and PRL release.

	- A-23187		+ A-23187 10 µM	
	cAMP	PRL	cAMP	PRL
Control	31 ± 3	173 ± 14	104 ± 8**	300 ± 22**
DA 1 uM	22 ± 2*	117 ± 11*	69 ± 10*	229 ± 13*
PF 1 uM	23 ± .8*	119 ± 5*	51 ± 15*	206 ± 12*
PF 10 uM	16 ± 1**	55 ± 12**	38 ± 2**	79 ± 9**

*= $p<0.05$; **= $p<0.01$ by ANOVA compared to the respective control.

These results indicate that an enhanced calcium influx activates cAMP accumulation in pituitary cells. The ability of CaI to stimulate PRL release may be, at least in part, due to the enhanced lactotroph cAMP content, because DA inhibited both basal and ionophore-stimulated cAMP accumulation and PRL release. The PF inhibition of ionophore-stimulated cAMP accumulation and PRL release suggests that calmodulin participates in these responses in the lactotroph. Thus, PRL release may be modulated by Ca^{2+}-calmodulin-dependent processes that positively interact with the cAMP system, and the inhibitory action of DA may be produced by interacting with this system. Supported in part by USPHS Grant CA-07535 (RMM), RCDA 1KO4 NS00601 and AM22125 (MJC).

18.28 DOPAMINE INHIBITS THE INCORPORATION OF $^{32}P_i$ INTO PHOSPHATIDYLINOSITOL OF ANTERIOR PITUITARY GLANDS. P.L. Canonico,* C.A. Valdenegro,* S.B. O'Dell* and R.M. MacLeod*. (SPON: J.H. Johnson). Dept. of Internal Medicine, University of Virginia School of Medicine, Charlottesville, VA 22908.

Dopamine (DA) has a major function to inhibit prolactin (PRL) secretion from anterior pituitary gland, whereas it does not seem to be directly involved in regulating secretion of other pituitary hormones. Neurotransmitters and peptide hormones have been found to increase phosphatidylinositol (PI) cleavage and turnover in a variety of cell types, including endocrine cells.

We studied the effect of DA and DA antagonists on $^{32}P_i$ incorporation into PI, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the anterior pituitary gland of female rats. Incubation of the glands for 30 min with 50 uCi/ml $^{32}P_i$ Na_2HPO_4, and DA at concentrations of 0.5, 5 and 50 µM caused a significant ($p<0.01$) reduction in the incorporation of $^{32}P_i$ into PI, while, in contrast, no change occurred in ^{32}P-PC or -PE labelling. This effect was also studied as a function of the incubation period. The inhibition produced by 0.5 uM DA was observable after 20 min (-47%), was maximal at 30 min (-55%) and was still present after 60-120 min (-41 and -35% respectively). These concentrations of DA caused rapid and significant inhibition in PRL release. Haloperidol and pimozide (50 nM) did not modify the incorporation of $^{32}P_i$ into PI, PC and PE by themselves, but completely abolished the inhibition of $^{32}P_i$ incorporation into PI produced by DA. The incorporation of $^{32}P_i$ into PI, PC and PE was also investigated in the pituitary gland of female rats injected with a specific inhibitor of catecholamine synthesis α-methyl-p-tyrosine (αMpT) (200 mg/kg i.p.) 2.5 hrs before sacrifice. Serum PRL levels in αMpT treated rats were considerably higher (641 ± 117 ng/ml) than in saline injected rats (16 ± 3). The glands from these animals, incubated for 30 min with $^{32}P_i$, showed a significant increase (+130%) in the incorporation of the radiolabelled P_i into PI, but not into PC or PE. When 0.5 uM DA was added in vitro to pituitary glands from αMpT treated rats, the stimulation of $^{32}P_i$ into PI induced by αMpT was completely inhibited. To verify whether the chronic effect of DA on the PI cycle is also present in vivo conditions, we utilized female rats bearing the transplanted PRL secreting tumor MtTW15. The high serum PRL levels produced by the tumor stimulate the synthesis and release of DA from tuberoinfundibular dopaminergic neurons which suppress pituitary gland prolactin synthesis and release. When these pituitary glands were incubated for 30 min with $^{32}P_i$, an accompanying inhibition (-44%) of incorporation into PI but not into PC and PE was observed in comparison with non-tumor bearing animals.

Our results suggest that the inhibition of PI turnover may represent a biochemical event related to the stimulation of pituitary DA receptors responsible for the inhibition of PRL secretion. (Supported in part by USPHS Grant CA-07535 from the National Cancer Institute)

18.29 LONG-TERM TREATMENT WITH ESTRADIOL ATTENUATES THE PROLACTIN-INDUCED ACTIVATION OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS. M.K. Pryal*, G.D. Riegle*, K.E. Moore and K.T. Demarest (SPON: J.E. Thornburg). Depts. of Pharmacol./Toxicol. and Physiol., Michigan State Univ., East Lansing, MI 48824.

Previous studies have demonstrated that short-term treatment with large doses of estradiol benzoate (25 µg/kg, sc for 3 days) stimulates the synthesis and turnover of dopamine (DA) in the terminals of tuberoinfundibular neurons in the median eminence (Endocrinology 106: 463, 1980). This action of estradiol is thought to be mediated by increased circulating levels of prolactin. The present studies were undertaken to compare serum prolactin concentrations and tuberoinfundibular DA neuronal activity in ovariectomized, estradiol-treated rats. The activity of these neurons was estimated by measuring the rate of DOPA accumulation in the median eminence 30 min after the administration of an inhibitor of aromatic L-amino acid decarboxylase (NSD 1015, 100 mg/kg, i.p.). Female rats, ovariectomized for two weeks, were implanted with silastic capsules containing estradiol benzoate, which maintained serum concentrations of this hormone in the range of 60-80 pg/ml. Following the implantation of the estradiol-containing capsule serum prolactin concentrations were markedly increased 6 and 18 days later. The rate of DOPA accumulation in the median eminence was significantly increased at 6 days but not at 18 days after the initiation of estradiol treatment. Interestingly, the concentration of DA in the median eminence declined at 6 days after estradiol treatment and a further decline was noted after 18 days despite the fact tha serum prolactin concentrations were still elevated. This suggests that long-term estradiol treatment in ovariectomized rats reduces the responsiveness of tuberoinfundibular DA neurons to prolactin. This was confirmed by direct experimentation. In sham-implanted rats, the rate of DOPA accumulation in the median eminence was increased 12 h after the intracerebroventricular infusion of prolactin (10 µg/10 µl); this effect was not seen in estradiol-treated rats. These results suggest that long-term treatment with estradiol attenuates the prolactin-induced activation of tuberoinfundibular DA neurons. (Supported by USPHS grant NS9174.)

18.30 ACUTE EFFECTS OF ESTRADIOL ON THE TURNOVER OF SEROTONIN IN DISCRETE BRAIN REGIONS AND ON PITUITARY HORMONE SECRETION. M.D. Johnson*, L.C. Terry, and W.R. Crowley, Dept. of Pharmacology, Univ. of Tennessee, Memphis, TN 38163 and VA Medical Center, Ann Arbor, MI 48105.

Central serotonin (5-HT) systems may participate in the regulation of luteinizing hormone (LH) and prolactin (PRL) secretion from the anterior pituitary gland. These studies employed analysis of 5-HT turnover and discrete 5-HT denervations to test whether the acute effects of estradiol on LH and PRL are mediated by changes in activity of specific 5-HT systems. Ovariectomized female rats received oil vehicle or estradiol benzoate (EB; 50µg) three hours prior to decapitation. Each hormone group was subdivided further so that one third received saline and the remainder received 75 mg/kg of the monoamine oxidase inhibitor, pargyline HCl, 15 or 30 min prior to decapitation. Plasma concentrations of LH, PRL, GH, and TSH were determined by double antibody radioimmunoassays. Brains were frozen, sectioned in a cryostat, and eight individual nuclei were microdissected. Concentrations of 5-HT and its metabolite, 5-hydroxyindole acetic acid (5-HIAA), were measured by liquid chromatography with electrochemical detection. In other animals, the content of LH releasing hormone (LHRH) was measured in the median eminence by radioimmunoassay.

Administration of EB produced a gradual decline of LH and rise of PRL within the three hour period but did not change GH or TSH, or median eminence levels of LHRH. While estrogen treatment did not change steady state levels of 5-HT, the hormone did elevate 5-HIAA concentrations, suggesting enhanced 5-HT release, in the medial preoptic, ventromedial and cortical amygdaloid nuclei. Furthermore, estrogen-treated animals showed a potentiation of the accumulation of 5-HT and enhanced decline of 5-HIAA following pargyline in these three nuclei, suggesting increased turnover. No changes of 5-HT turnover were noted in the median eminence or in the stria terminalis, periventricular, dorsomedial or lateral amygdaloid nuclei. In further studies, whole brain 5-HT depletion produced by systemic p-chlorophenylalanine (400 mg/kg) did not prevent either the decrease of LH or increase of PRL after EB, and also did not affect GH or TSH. However, preliminary observations in desmethylimipramine-pretreated, ovariectomized rats show that microinjections of the 5-HT neurotoxin, 5,7-dihydroxytryptamine, into the cortical amygdala attenuated the rise of PRL observed after EB, without modifying the decrease of LH or basal levels of either hormone.

These results suggest that estradiol acutely elevates PRL, in part, via increased serotonergic activity in the cortical amygdala, and possibly, other discrete nuclei.
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18.31 LOCALIZATION OF SEROTONIN AND SOMATOSTATIN FIBERS IN THE RAT ADENOHYPOPHYSIS. Karin N. Westlund and Gwen V. Childs*, Department of Anatomy, The University of Texas Medical Branch, Galveston, Texas 77550.

The anterior pituitary contains receptors for both serotonin and somatostatin (Johns et al. and Enjalbest et al., Endocrinol. 110, 1982). In addition, the pituitary contains measurable amounts of serotonin and somatostatin. The objective of this study was to determine if serotonin or somatostatin fibers could be traced to specific sites in the adenohypophysis. We initially compared a variety of fixation, embedding and immunocytochemical staining techniques. Male rats were perfused with warm saline, followed by cold buffered 4% paraformaldehyde (PF), and then cold buffered 30% sucrose. Horizontally or sagittally cut (15µm) frozen sections were stained with one of two immunocytochemical methods: (1) 1:20,000 dilution of anti-serotonin or 1:5,000 dilution of antisomatostatin and the peroxidase antiperoxidase (PAP) technique. The PAP complex was localized with diaminobenzidine chloride (DAB) intensified with nickel ammonium sulfate (Hancock, Histochem. Abst., 1982), or (2) 1:75,000 dilution of antiserotonin or 1:25,000 dilution of antisomatostatin and the avidin biotin peroxidase complex (ABC) technique (Childs and Unabia, J. Histochem. Cytochem. 30, 1982) intensified with nickel ammonium sulfate. A second set of male rat pituitaries were immersion fixed in 1% glutaraldehyde (Gl) or 4% PF and embedded in Araldite 6005. A third set of pituitaries was freeze fixed in the Boyne Rapid freezing apparatus, followed by freeze drying *in vacuo*, and embedding in Epon (Dudek et al., J. Histochem. Cytochem. 30, 1982). Thick and ultrathin plastic sections were cut and stained for serotonin or somatostatin with the ABC technique (Childs and Unabia, J. Histochem. Cytochem. 30, 1982). In the frozen, unembedded sections, fibers and varicosities staining for serotonin and somatostatin were observed to enter the rostral zone or "nasale Umschlagszone" of the adenohypophysis with blood vessels and split into fine, varicose fibers in deeper portions of the anterior lobe, in agreement with ultrastructural studies of Kurosumi and Kobayashi (Arch. hist. jap. 43, 1980). Fibers staining either for serotonin or somatostatin were along the periphery of the anterior and posterior lobes especially at the poles and along the surface of the anterior lobe adjacent to the cleft. Fine, varicose fibers were also scattered throughout the intermediate lobe. In the freeze fixed, plastic embedded sections, staining for serotonin appeared in collections of fibers or processes extending from the periphery of the gland into the first 2-3 cell layers. These studies suggest that fiber systems containing serotonin or somatostatin innervate the adenohypophysis and can be detected with most sensitive methods (ABC-Intensified DAB). Supported by the Kempner Foundation.

18.32 EFFECT OF SEROTONIN AGONISTS AND PRECURSORS ON PITUITARY HORMONE SECRETION IN MAN. H.Y. Meltzer, N.Nash*, G. Manov*, B.J. Tricou*, and V.S. Fang*. Departments of Psychiatry and Medicine, University of Chicago Pritzker School of Medicine and the Illinois State Psychiatric Institute, Chicago, IL. 60637

The role of serotonin (5-HT) in the secretion of prolactin (PRL), growth hormone (GH) and cortisol in man has been quite controversial. The precursor of 5-HT, 5-hydroxytryptophan (5-HTP), has been most frequently employed as a stimulus to examine this question, with very mixed results. Quipazine, a direct acting 5-HT agonist, has been reported to stimulate cortisol secretion, but not that of PRL or GH, in normal volunteers.

We have administered the indole hallucinogen, N,N-dimethyltryptamine (N,N-DMT), 0.7 mg/kg intramuscularly (i.m.) or saline, to 8 volunteers, after pretreatment with placebo or cyproheptadine (Cyp), a putative 5-HT antagonist, 4 mg t.i.d. for 3 days. Experimental days were at least 1 week apart, the conditions were randomly presented to each subject, and the experimenters were blind concerning the pretreatment and injection schedule. Three of the subjects were also tested in a fifth condition: pretreatment with haloperidol (Hal), 0.5 mg, i.m., 60 min before DMT. Subjects had an indwelling venous catheter to permit multiple blood samples from 30 min before to 3 hr after DMT.

Serum PRL, GH and cortisol increased in most but not all subjects after placebo-DMT. The increase in PRL and cortisol peaked 30 min after DMT whereas GH peaked at 60 min. Cyp pretreatment blocked the GH increase but had minimal effect on PRL and cortisol. Hal did not affect the GH increase. Cyp and Hal had only slight effects on the psychotomimetic effects of DMT.

In a separate study, 5-HTP, 200 mg p.o., was administered to 10 normal volunteers, unmedicated patients with mania (N=10) or depression (N=10) and to the latter, following two-three weeks treatment with lithium or tricyclic antidepressants (TAD). Results to date indicate that 5-HTP tended to increase serum cortisol levels in the patients with affective disorders but not the normal controls. Treatment with TAD appeared to diminish the cortisol response to 5-HTP whereas lithium treatment appeared to enhance the response. However, the number of subjects studied to date is insufficient for these findings to be conclusive. 5-HTP had no consistent effect on PRL or GH levels. Supported, in part, by USPHS MH 30059.

18.33 DIURNAL CHANGES IN THE UPTAKE OF SEROTONIN AND NOREPINEPHRINE IN NUCLEAR REGIONS DURING THE ESTROUS CYCLE AND THE EFFECTS OF NEUROTOXIN LESIONS ON ESTROUS CYCLICITY. Donald C. Meyer, and Jyoti Singh, Department of Physiology and Biophysics, University of Louisville School of Medicine, Louisville, Kentucky 40292.

The temporal pattern of hypothalamic and limbic aminergic activity during the estrous cycle in the regularly cycling female Sprague Dawley rat has been investigated by measuring the changes in serotonergic and noradrenergic neuronal uptake during the regular estrous cycle (0500-1900 L). Significant changes ($P < .05$) in the saturation uptake of ^3H 5-HT and ^3H NE were recorded in the suprachiasmatic nuclei (SCN) at 1200 hours on proestrus. Significant changes ($P < .05$) in the uptake of ^3H 5-HT in the preoptic area (POA) were found at 1200 hours on both diestrus and proestrus while ^3H 5-HT showed a significant peak ($P < .05$) in the median eminence (ME) only during proestrus. In the amygdala (AMYG) ^3H 5-HT uptake was significantly different at 1200 hours on diestrus and proestrus, while ^3H NE uptake was significant ($P < .05$) at 1200 hours only during proestrus. During these experiments the plasma proestrus luteinizing hormone surge as measured by radioimmunoassay occurred at 1700 hours.

To further assess the role of serotonin in ovulation and estrous cyclicity, 5,7-dihydroxytryptamine lesions (5,7-DHT) were placed in the SCN, POA, ME, and AMYG. 5,7-DHT was stereotaxically injected thru a 30 gauge needle in concentrations of 5-10 µg/ul over a time period of one minute. In the SCN 4 or 5 day estrous cyclicity was interrupted by diestrus for average periods of up to 15 days; in the POA for periods of 10 days; in the median eminence for periods of 13 days; and in the amygdala for periods of up to 6 days. Following these periods of acyclicity rats resumed normal cycles.

These lesion effects and patterns of uptake suggest a common timing mechanism utilizing both serotonin and possibly norepinephrine for neuroendocrine control. Lesions with the serotonin neurotoxin disturb estrous cyclicity in all four brain regions, but the greatest delays occur in the SCN and ME, regions which are particluarly critical to intrinsic neuroendocrine rhythms. The patterns of change in reuptake capacity in all four regions occur 3 hours prior to the critical period for the plasma LH surge and may be an important mechanism for many types of neuroendocrine events including ovulation.

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18.34 ACUTE ESTROGEN NEGATIVE FEEDBACK: TEMPORAL CORRELATION BETWEEN ESTRADIOL UPTAKE AND NUCLEAR TRANSLOCATION AND NOREPINEPHRINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE METABOLISM IN DISCRETE PREOPTIC AND HYPOTHALAMIC REGIONS. C.A. Johnston, M. Tesone* and A. Negro Vilar, Dept. of Physiology, Univ. of Texas Health Science Center, Dallas, TX 75235.

This study was designed to examine the cellular mechanisms by which estradiol exerts its negative feedback effects upon gonadotropin secretion in gonadectomized animals. The uptake of estradiol by cytosol receptors and the subsequent translocation of the estradiol receptor to the nucleus in preoptic (POA) and hypothalamic (HYP) regions were correlated with changes in neuronal activity of norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT)-containing neurons of the suprachiasmatic (SCN), medial preoptic (MPO) anterior (AN1) and posterior (AN2) arcuate and dorsomedial (DMN) nuclei, as well as in the median eminence (ME), in ovariectomized (OVX) rats. Ten days following OVX, rats received either oil or estradiol benzoate (Eb) (20 µg/rat, s.c.) a treatment which resulted in a marked drop in the castration-induced elevation of serum luteinizing hormone (LH) concentrations within 3 h of injection. Cytosolic (CER) and nuclear (NER) estrogen receptors were measured using an exchange assay at various times following Eb injection in POA, HYP and anterior pituitary (AP) tissues. The activity of the NE, DA and 5-HT neuronal systems in discrete regions within those brain areas was estimated concurrently by measuring the concentrations of NE, DA, and 5-HT and their major metabolites: 3-methoxy-4-hydroxyphenylglycol (MHPG), 3-4-Dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) respectively, utilizing high performance liquid chromatography with electrochemical detection. Eb stimulated nuclear translocation of the receptor by 3h in both HYP and AP, while NER levels in POA were already increased at 1h, slightly preceding the first detectable drop in serum LH concentrations. Following OVX, NE metabolism increased in the SCN, MPO and DMN, and decreased in the AN2 when compared to diestrous controls. These changes were completely reversed 3 h after Eb, at a time when effective increases in NER had taken place. Similarly, changes in DA and 5-HT concentrations (but not metabolism) observed after OVX were also reversed by Eb. The results indicate that acute nuclear translocation of estradiol receptors in POA and HYP effectively modifies NE metabolism in several discrete nuclei reportedly involved in the regulation of LHRH/LH release, support the concept that rostral noradrenergic neuronal systems are involved in the negative feedback of estrogen upon LH secretion and suggest the possibility that some NE neurons within the mediobasal hypothalamus may play an inhibitory role in the regulation of LH release. Supported by NIH 09988-06, Project III.

18.35 ANALYSIS OF STEROID-MONOAMINE FEEDBACK INTERACTIONS IN DISCRETE BRAIN REGIONS USING AS A MODEL THE MONOSODIUM GLUTAMATE (MSG) LESIONED RAT. M. Tesone*, C.A. Johnston and A. Negro-Vilar, Dept. of Physiology, Univ. of Texas Health Science Center, Dallas, Tx 75235.

Neurodegenerative lesions induced by neonatal treatment with MSG elicit a number of endocrine and behavioral deficiencies such as hypogonadism, sluggish gonadotropin responses to gonadectomy, irregular cycling in females and impaired lordotic responses. Previous studies from our laboratory (Endocrinology 110: 835, 82) indicate that the mechanism(s) that normally triggers LHRH release is defective in these animals. The present studies were undertaken to determine whether the reported deficiencies in monoamine input to selected preoptic and hypothalamic regions affect steroid receptor kinetics in those areas. Moreover, steroid negative and positive feedback effects were evaluated and the responses compared to those of normal controls. Adult female rats, injected neonatally with either MSG or hypertonic saline were used for these experiments. Analysis of dopamine, norepinephrine and serotonin metabolism by HPLC with electrochemical detection, revealed substantial decreases in the metabolism of all three amines in the arcuate nucleus, as well as reduced dopamine and serotonin metabolism in the suprachiasmatic nucleus. Measurement of estradiol receptors in preoptic (POA), hypothalamic (HYP) and anterior pituitary (AP) tissues from MSG-treated and control rats killed during diestrus or 2 weeks after ovariectomy (OVX), revealed no difference in either cytosolic (CER) or nuclear (NER) estrogen receptor levels. Priming of OVX rats with estradiol for 2 days induced similar levels of NER for both groups in all three areas. Evaluation of cytosolic progestin receptors (CPR) using R-5020 as ligand, revealed no differences between control and MSG unprimed-OVX or estradiol-primed rats. Both the negative and positive feedback effects of estradiol, as well as pituitary responsiveness to LHRH were not significantly impaired in MSG rats. These results indicate that the MSG-induced damage to dopaminergic, serotonergic and noradrenergic elements within several preoptic and hypothalamic nuclei does not impair estrogen and progestin receptor kinetics; nor does it prevent adequate negative or positive steroid feedback responses, if appropriate steroid regimens are employed. Supported by NIH 09988-06, Project III.

18.36 STRAIN DIFFERENCES IN NUMBER OF HYPOTHALAMIC DOPAMINE NEURONS: RELATIONSHIP TO PITUITARY STORAGE AND RELEASE OF PROLACTIN H. Baker, A.F. Sved, S. Alden*, L.W. Tucker* and D.J. Reis. Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

There are fewer dopamine (DA) neurons in all dopamine cell groups of the hypothalamus of mice of the BALB/cJ strain as compared to those of the CBA/J strain. Some of these neurons, specifically the tuberoinfundibular (TI) DA neurons (A12 group), act to inhibit the synthesis and release of prolactin (PRL) from the anterior pituitary (AP). We sought to determine whether the differences between the number of DA neurons was reflected by an appropriate variation in the content and release of PRL in male mice of the two strains. PRL, measured by radioimmunoassay, was higher in the serum in CBA/J as compared with BALB/cJ mice (9.8 ± 0.6 and 7.8 ± 0.6, $\bar{x}$ ng/ml + SEM, respectively; $n = 10$; $p<.05$) and in the pituitary (CBA/J, 2.03 ± 0.06; BALB/cJ, 1.62 ± 0.05; $\bar{x}$ µg/gland + SEM; $n = 10$; $p<.05$); thus, the strain with more DA neurons has less PRL. To establish the cytochemical basis for the strain differences in pituitary PRL content, pituitaries from mice of each strain were immunocytochemically stained with antibodies to PRL using the peroxidase antiperoxidase method. Sections were examined using a computer assisted image analysis system. Quantitative immunocytochemical staining was carried out under linear reaction conditions with diaminobenzidine (DAB) as the chromagen (Benno et al., Br. Res., 1982). The average optical density (AOD) of the DAB reaction product, a measure of the density of staining averaged over an entire tissue section, was determined at low power (2.5x) throughout the pituitary. AOD was greater in CBA/J than BALB/cJ mice (0.054 ± 0.003 vs. 0.036 ± 0.002, $\bar{x}$ OD units + SEM, $n = 4$-5; $p<.05$). This difference in AOD could result from either greater staining intensity in the same number of pituitary cells or differences in the total number of cells containing PRL. Since the AOD of the darkest staining tissue, measured at high power (40x), did not differ significantly between strains, the variation in AOD was not a result of differences in staining intensity in similar numbers of cells. However, with comparable staining conditions, within any field there were more cells containing PRL in CBA/J than in BALB/cJ mice. The average number of acidophils stained by the Pearse trichrome method, representing lactotrophs (containing PRL) and somatotrophs (containing growth hormone), did not differ between strains. Thus, either CBA/J mice have more lactotrophs or some lactotrophs in BALB/cJ mice do not contain sufficient PRL to be demonstrable with the antibody technique. The strain difference in the number of PRL-containing cells in the AP suggests that genetic variation in the number of hypothalamic TI DA neurons is reflected by appropriate functional differences in the storage and release of PRL from the pituitary.

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18.37 THE EFFECT OF HALOPERIDOL ON OVINE REGIONAL CEREBRAL AND MEDIAN EMINENCE BLOOD FLOW. J. Townsend*, R. M. Bryan, R. W. Brennan*, R. B. Page*. Dept. of Surgery (Div. of Neurosurgery), Dept. of Medicine (Div. of Neurology), Dept. of Physiology and Dept. of Anatomy, M. S. Hershey Medical Center of the Pennsylvania State University, Hershey, PA 17033.

Dopaminergic axons terminate near blood vessels in the median eminence. To test the hypothesis that dopamine regulates median eminence blood flow, we measured regional cerebral and median eminence blood flow before and after infusion of haloperidol, a dopamine antagonist. Five female non-pregnant sheep were anesthetized with pentobarbital, intubated, and passively ventilated. Right and left femoral arteries were cannulated for injecting drugs, withdrawing blood, and monitoring blood pressure. After a left thoracotomy, a double lumen cannula was inserted into the left atrium by passing it directly through the atrial wall and was used for injecting microspheres. Blood gases and pH were measured from arterial blood samples and maintained within normal range by adjusting the respirator. Blood flow was measured twice in each sheep, once before and once after an I.V. infusion of haloperidol (0.5 mg/kg over 30 minutes). After haloperidol infusion blood flow decreased in all regions measured: subcortical white 17%, cortical gray 18%, neural lobe 21%, caudate nucleus 23% and median eminence 33% ($P < .02$). Renal blood flow fell 29% on average. Mean arterial blood pressure did not change with haloperidol infusion. Since dopamine is locally released in the median eminence under physiological conditions and haloperidol, a dopamine antagonist, significantly decreased median eminence blood flow, we conclude that the local release of dopamine is a mechanism for regulation of blood flow in the median eminence.

18.38 THE RELEASE OF DOPAMINE (DA) INTO HYPOPHYSIAL PORTAL BLOOD IN THE MALE HYPERPROLACTINAEMIC RAT. A. Brar*, V. Kapoor*, A. McNeilly*† G. Fink (SPON: D.W. Lincoln). MRC Brain Metabolism Unit, Department of Pharmacology, 1 George Square, Edinburgh, U.K., and †MRC Reproductive Biology Unit, 37 Chalmers Street, Edinburgh, U.K.

Studies in vitro and the measurement of DA in the hypothalamus have suggested that prolactin (PRL) may increase DA release into hypophysial portal vessel blood and thereby invoke a negative feedback system. We have investigated this possibility by measuring DA in hypophysial portal vessel blood in control adult male rats and in male rats made hyperprolactinaemic by transplanting two pituitary glands under the kidney capsule. Hypophysial portal vessel blood was collected (Fink, G. and Jamieson, M.G., J. Endocrinol. 68: 71, 1976) for 30 min before, during and after the application of an electrical stimulus (30 sec trains of biphasic square wave pulses, 60 Hz, 1mA peak to peak and 1 mA duration) to the median eminence. The portal blood was collected into Trasylol (20,000 KIU/ml) and EDTA (5.4 mM l^{-1}) at 4°C. The concentrations of DA and DOPAC were measured in plasma extracts using HPLC with electrochemical detection (Plotsky, M. et al, Endocrinology 102: 1887, 1978).

Concentration of DA and DOPAC in hypophysial portal plasma (ng/ml, mean ± S.E.M.)

	Pre-stimulation	Stimulation	Post-stimulation
CONTROLS (n=6)			
DA	3.62 ± 0.55	2.66 ± 0.98	2.20 ± 0.07
DOPAC	7.20 ± 0.24	4.60 ± 0.28	7.00 ± 1.98
HYPER-PRL (n=5)			
DA	4.48 ± 0.34	4.56 ± 0.39	-
DOPAC	9.47 ± 1.32	12.20 ± 1.10	-

The table shows that DA and DOPAC levels were slightly higher in the hyperprolactinaemic group than in the control group, although the differences were only significant in the sample collected during stimulation. These results suggest that long-term elevations in plasma PRL (approx. 120 ng NIH-RP-1/ml) elevate the turnover rate of the tuberoinfundibular DA system without significantly affecting the DA concentration in portal blood. Furthermore, despite a 47% depletion of DA concentration in the median eminence (ME) produced by long-term hyperprolactinaemia (Simpkins, J.W. et al, Life Sci. 30: 1249, 1982), electrical stimulation of the ME can augment the PRL-induced elevation of DA turnover while keeping DA concentration constant.

18.39 DOMPERIDONE SELECTIVELY ALTERS THE RATE OF DOPAMINE SYNTHESIS IN THE MEDIAN EMINENCE AND POSTERIOR PITUITARY IN THE RAT. J.M.Farah, Jr.*, K.T.Demarest, G.P.Mueller, and K.E.Moore (SPON: D.J. Pettibone). Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

Blockade of dopamine receptors in the central nervous system with haloperidol (HAL) is associated with increased dopamine synthesis which is thought to accompany increased dopaminergic neuronal activity. Another dopamine receptor antagonist, domperidone (DOM), is frequently used to study dopaminergic physiology outside the central nervous system because of the reported inability of systemically administered DOM to cross the blood-brain barrier (Laduron & Leysen, 1979, Biochem. Pharmacol. 28:2161). To further characterize the peripheral versus possible central effects of DOM, we compared the effects of DOM with those of HAL on the rate of dopamine synthesis in dopamine neurons thought to terminate outside the blood-brain barrier (i.e., in the median eminence and posterior pituitary) and those which are located within the blood-brain barrier (i.e., striatum, nucleus accumbens, and olfactory tubercle). Rate of dopamine synthesis was estimated in adult, male, Long-Evans rats by the accumulation of DOPA 30 min after administration of a DOPA-decarboxylase inhibitor (NSD 1015, 100 mg/kg, ip). DOPA was measured by radioenzymatic assay of 0.2 N $HClO_4$ tissue extracts from animals treated with 2.5 mg/kg DOM or HAL sc either 2, 8, or 16 h before sacrifice; efficacy of dopamine receptor blockade in the periphery was estimated by plasma levels of pituitary hormones thought to be tonically inhibited by dopamine (prolactin, α-melanotropin, and β-endorphin-like immunoreactivity).

DOM, like HAL, increased DOPA accumulation in the median eminence only after a delay of 16 h, and increased posterior pituitary DOPA accumulation 2, 8 and 16 h after administration. Unlike HAL, which increased DOPA accumulation in the striatum, nucleus accumbens, and olfactory tubercle 2, 8, and 16 h after injection, DOM had no effect on dopamine synthesis in either the striatum or nucleus accumbens, and only moderately increased DOPA accumulation in the olfactory tubercle. In non-NSD-treated rats, DOM increased plasma levels of α-melanotropin and β-endorphin-like immunoreactivity 2 h and 4 h after injection, and plasma prolactin was markedly increased over control values 1 h after DOM and remained elevated for up to 16 h. The present findings demonstrate that DOM selectively increases the rate of dopamine synthesis in dopamine neurons which terminate in the median eminence and posterior pituitary but not those which terminate in the striatum, nucleus accumbens and olfactory tubercle. (Supported by USPHS Grant NS 15911 & USUHS Grant RO7632)

18.40 COMPARISON OF IONIC CURRENTS IN VOLTAGE-CLAMPED PITUITARY TUMOR CELLS IN CULTURE. J. M. Dubinsky and G. S. Oxford*. Neurobiology Program and Physiology Department, University of North Carolina, Chapel Hill, NC 27514.

Ionic currents have been identified in voltage-clamped cells from the GH3 and GH4 rat pituitary tumor cell lines. Individual cells (10-17um) were voltage-clamped using the new patch clamp technology (Hamill et al., Pfluger's Arch. 391:85, 1981). Patch pipets were gently apposed to the cell membrane and with the application of suction a "gigohm" seal was formed between the pipet and the cell. Additional suction ruptured the membrane under the pipet and permitted intracellular recording and internal dialysis of the cell cytoplasm. Resting potentials averaged -43.0 ±13.6mv. Membrane resistance was in the range of 0.5 - 5 Gohm. When voltage steps were applied from -50mv to -70mv holding potentials, both inward and outward currents were present. Series resistance was partially compensated and capacitative transients and leakage currents were corrected by computer subtraction of hyperpolarizing control pulses.

A fast inward current which inactivated had the voltage- and time-dependence of Na current and was blocked by tetrodotoxin (TTX). For large depolarizations this current became outward and its reversal potential shifted as expected from the Nernst relation for changes in external [Na^+]. Another inward current which did not appear to inactivate had a slower onset, was blocked by external Co^{++}, Zn^{++}, Mn^{++}, and Cd^{++}, and was sensitive to the external [Ca^{++}]. Ba^{++} could substitute for Ca^{++}as the current carrier.

The outward current appeared to have three components: (a) one sensitive to external 4-aminopyridine (4AP) and internal Cs^+, (b) one sensitive to external Co^{++}, internal Cs^+, or EGTA, and (c) one insensitive to all of these agents. K^+ appears to be the major ion carrier of the first two types of outward current. Inactivation of the first type of outward current was observed during 185ms pulses and resulted in a use-dependent decrease in outward current with repeated pulses. The second type of outward current was slower in onset and continued to increase steadily with time.

Thus it appears that the GH3 and GH4 cell membranes possess several voltage-dependent ionic channels selective for sodium, calcium, and potassium as well as a calcium-activated K channel. While both cell types contained all of these currents, the relative proportions of Na and Ca currents and the two types of outward K currents varied greatly between the GH3 and GH4 cell lines. The functional implications of these findings will be discussed.

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18.41 THREE-DIMENSIONAL RECONSTRUCTION OF THE MORPHOLOGY OF NERVE-GLIA RELATIONSHIPS IN THE NEURAL LOBE OF THE PITUITARY. W. Gregory, M. R. Park and C. D. Tweedle. Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Previous reports have shown that pituicytes, specialized glial cells of the neural lobe of the hypophysis, can encapsulate neurosecretory nerve endings and that the extent of the glial containment is a function of rate of hormone release (Neurosci. 5, 661-667; Brain Res. 192, 555-559). As the surrounding of neurosecretory nerve endings is a plastic process, it seemed profitable to precisely reconstruct the three-dimensional arrangement of these elements from serial electron micrographs.

A system of programs has been developed on the PDP-11 computer for the reconstruction, display, and analysis of morphological data. From serial electron micrographs, the outline of each profile considered to be of interest is entered into the computer as a series of x, y coordinates, using a digitizing pad (Bitpad). The third coordinate (z) is a function of section number and is entered by the operator. The mass of x, y, z coordinates can be manipulated by the computer, rotated according to the rules of analytical geometry, and drawn by a graphics plotter. A critical step in reconstruction is the accurate placement of fiduciary marks on each micrograph which establish the origin and orientation of the Cartesian coordinate system used and which orient adjacent serial sections to each other. This step is performed manually. Experience has shown that the eye and judgement of the investigator are the most efficient for detecting and aligning ultrastructural features useful in placing these reference marks.

The membrane and cytoplasm of that portion of a pituicyte adjacent to a neurosecretory ending can be seen, in the serial reconstruction, to be thrown first up into ridges which become progressively higher and finally completely encircle the nerve ending. Additional ridges can form from the thin rim of cytoplasm surrounding one nerve ending to engulf another. Numerous neurosecretory endings can be contained within a localized region of a single pituicyte. The geometric arrangement of membrane around neurosecretory processes cannot be topologically described as a single unbroken surface. This is evidenced by the presence of fenestrations along sheaths encircling a neurosecretory process and the observation of processes which enter and then leave an ensheathing portion of pituicyte. To have formed complex geometrical arrangements, both fusion and cleavage of adjacent cytoplasmic extensions must have taken place. The observation of the transition in space which results in the encircling of a process, though static, suggests a means by which neurosecretory processes are actively engulfed. (Supported by NIH Grant RR 05772.)

18.42 PRESENCE OF CRF-LIKE IMMUNOREACTIVITY IN HYPOPHYSIAL PORTAL BLOOD. D.M. Gibbs and W. Vale. Dept. Repro. Med., UCSD Sch. Med., La Jolla, CA 92093 and The Salk Institute, La Jolla, CA 92138.

Recently a 41 amino acid ovine hypothalamic peptide with potent CRF activity in vivo and in vitro has been isolated, characterized, and synthesized (Vale et al., Science 213: 1394, 1981). We now describe the presence in rat hypophysial portal plasma of a substance which is bound by an antiserum raised against synthetic ovine CRF (oCRF).

Hypophysial portal blood was collected from Nembutal-anesthetized male rats by the parapharyngeal technique of Porter. Immunoreactive CRF was extracted from plasma by adsorption to ODS silica cartridges (Bond Elut) and elution with acetonitrile-TEAF. Immunoreactivity was measured in an RIA consisting of antiserum raised against oCRF, oCRF standard and iodinated oCRF trace. The limit of detection was 25 pM.

The mean concentration of CRF-like immunoreactivity (CRF-LI) in portal plasma from 9 rats was 104.9 ± 9.7 pM ($\bar{x}$ + SEM) while the mean concentration in peripheral plasma was $27.5 ± 0.8 pM ($p < 0.0001$). Inhibition curves for synthetic ovine CRF, highly purified rat CRF, and portal blood CRF-LI were parallel, but the affinity of the antiserum for rat CRF was about 5-fold less than for ovine CRF. Therefore, portal blood CRF-LI levels expressed in terms of oCRF standard were probably underestimated by about 5 times.

An amount of oCRF equivalent to the concentration of CRF-LI found in rat portal blood has been shown to produce a 300% increase in ACTH secretion by pituitary cells in primary culture. This suggests that the concentration of CRF-LI in rat portal blood is sufficient to stimulate ACTH secretion and supports the hypothesis that oCRF, or a very similar molecule in the rat, is a physiologic hypothalamic releasing factor.

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18.43 CORTICOTROPIN RELEASING FACTOR (CRF)-INDUCED STIMULATION OF PROTEIN CARBOXYLMETHYLATION AND ACTH RELEASE IN MOUSE PITUITARY CELLS. S. Heisler*, V.Y.H. Hook*, and J. Axelrod (SPON: L. Larochelle). Section on Pharmacology, Laboratory of Clinical Science, NIMH, Bethesda, MD 20205 and Unite de Bioregulation Cellulaire et Moleculaire, Centre Hospitalier de 'Universite Laval, Quebec G1V 4G2 (SH)

Hormonal stimulation of several secretory organs is associated with an increase in the formation of protein methyl esters. Methyl groups can be transferred from S-adenosylmethionine to free carboxyl groups of protein substrates by the enzyme protein carboxylmethylase. Neutralization of negative charges of substrates localized on the surface of secretory vesicles could increase the probability of collision between the vesicles and the negatively charged plasma membranes thereby promoting intermembrane fusion and exocytotic secretion. The involvement of the protein carboxylmethylation reaction in CRF-stimulated ACTH secretion was investigated in the At-T-20/D16-16 mouse clonal pituitary cell line. Protein carboxylmethylation activity and ACTH secretion were both increased as a function of the concentration of extracellular CRF. Methylation was maximally increased by 40% and ACTH secretion by 400% at 10^{-8}M CRF. The half-maximal concentration for ACTH release and carboxylmethylation was about 3nM. CRF-stimulated ACTH release and protein carboxylmethylation activity were linear and temporally parallel for 50 to 90 minutes in the AtT-20 cells. The 21-methoxy derivative of CRF (10^{-7}M) had a similar effect on protein carboxylmethylation and ACTH release as 10^{-8}M CRF. The C-terminal free acid of CRF had no effect on either ACTH secretion or formation of protein methyl esters (10^{-9} to 10^{-6}M). A combination of 3-deazaadenosine and L-homocysteine thiolactone, inhibitors of the carboxylmethylation reaction, reduced CRF-stimulated carboxylmethylation by 55% and CRF-induced ACTH secretion by 37%. Dexamethasone (10^{-9} and 10^{-8}M; 20 hr pretreatment), known to inhibit both ACTH secretion and synthesis, had a marked inhibitory effect on ACTH release in the CRF-stimulated cells (80-90% reduction). Protein carboxylmethylation was less markedly, but significantly affected by dexamethasone (50% reduction). Post-translational modification of protein structure by protein carboxylmethylation appears to be a functionally important feature of hormone-stimulated secretion from several endocrine and exocrine glands. The data supports a similar relationship between protein carboxylmethylation and ACTH secretion in mouse pituitary cells.

18.44 EFFECT OF CORTICOTROPIN-RELEASING FACTOR ON PHOSPHOLIPID METHYLATION AND ACTH SECRETION IN MOUSE PITUITARY TUMOR CELLS. V.Y.H. Hook*, S. Heisler*†, and J. Axelrod. Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Md. 20205 and †Unite de Bioregulation Cellulaire et Moleculaire, Centre Hospitalier de l'Universite, Laval, Quebec, Canada G1V 4G2

Our laboratory has shown that the 41-residue synthetic ovine corticotropin releasing factor (CRF) stimulates ACTH and β-endorphin release in AtT-20/D16-16 mouse pituitary tumor cells. The response of these cells to CRF makes this homogeneous cell population a good model for investigating the biochemistry of CRF-receptor mediated molecular events required for ACTH and β-endorphin secretion. Phospholipid methylation of phosphatidylethanolamine to phosphatidylcholine with S-adenosylmethionine as methyl donor has been shown in several cellular systems to be involved as a possible membrane transduction mechanism for receptor-induced events. CRF increased phospholipid methylation by 30-50% at concentrations (10^{-9} to 10^{-8}M CRF) which also stimulated ACTH secretion. Both processes increased linearly with time up to 90 minutes incubation with CRF. The methionine sulfoxide derivative of CRF was less potent than CRF in stimulating both phospholipid methylation and hormone secretion, and the COOH-terminal free acid derivative of CRF had no effect on either process. CRF-induced increases in phospholipid methylation and ACTH secretion were reduced when cells were treated with the phospholipid methyltransferase inhibitors 3-deazaadenosine and L-homocysteine thiolactone. These CRF-stimulated effects were also blocked by the glucocorticoid dexamethasone. We have found that the AtT-20 cells also show changes in cAMP and protein carboxylmethylation in response to CRF. These findings suggest that phospholipid methylation in conjunction with other biochemical processes may be involved in CRF-receptor mediated stimulation of ACTH release.

18.45 FAILURE OF NEUROACTIVE AMINO ACIDS TO INFLUENCE LUTEINIZING HORMONE RELEASE FROM ISOLATED RAT OR MONKEY PITUITARY. J. TAL*, M. T. PRICE and J.W. Olney (SPON: R. Burde). Dept. of Psychiatry, Washington Univ. Sch. Med., St. Louis, MO 63110.

Glutamate (Glu) and certain of its structural analogs excite central neurons when tiny amounts are applied iontophoretically to their dendritic or somal surfaces. When larger amounts are applied, neurons are destroyed, apparently by an excitatory mechanism. Systemic administration of these "excitotoxic" agents results in destruction of neurons in brain regions that lack blood brain barriers such as the arcuate-median eminence (AH-ME) region of the hypothalamus. We have shown in adult male rats that subcutaneous (sc) injection of a subtoxic dose of glutamate (Glu) or N-methyl-aspartate (NMA), a potent excitotoxic analog of Glu, results in a rapid (7 1/2 min) tenfold increase in serum luteinizing hormone (LH). Pretreatment with sc GABA prevents NMA-induced LH release as does removal of neurons from AH-ME by sc Glu treatment in infancy. These findings suggest that the LH releasing action of Glu or NMA may involve excitation by these agents of AH neurons with consequent secretion of luteinizing hormone releasing hormone (LHRH) into the portal system. Consistent with this hypothesis, Schainker et al. (Brain Res., 184:425, 1980) reported that LH levels remain unchanged in isolated rat hemipituitaries incubated with NMA. R.C. Wilson and E. Knobil (personal communication) recently demonstrated that intravenous administration of NMA (15 mg/kg) to adult female rhesus monkeys induces a rapid elevation of serum LH and follicle stimulating hormone. Here we report that neither NMA nor Glu causes LH release from in vitro preparations of either rat or monkey pituitaries.

Hemipituitaries from male adult rats or 1/8 anterior pituitary from male pigtail monkeys were allowed to equilibrate at 37° for 30 min in constantly oxygenated Gibco medium #199. Incubation fluid was then replaced with fresh medium with added test agents and pituitaries were gently oscillated for 3 hr. LH was assayed by radioimmunoassay and agents were tested in concentrations from 10^{-8} to 10^{-3}M with or without LHRH. The only pituitary preparations from either rat or monkey that had significant elevations of LH were those that included LHRH; Glu, NMA or GABA, when incubated either with or without LHRH, failed to influence LH release. Our findings suggest that excitatory amino acids release LH not by a direct action at the pituitary level but by acting through AH-ME neurons which control LHRH secretion into the portal vascular system. Moreover, a suprasellar locus of action apparently also characterizes the inhibition by GABA of NMA-induced LH release. Supported by USPHS grants NS-09156, DA-00259 and RSA MH-38894 (JWO).

18.46 PULSATILE LH AND FSH SECRETION IN LONG-TERM OVARIECTOMIZED RATS: EVIDENCE FOR DIFFERENTIAL CONTROL MECHANISMS. T.P. Condon*, D.I. Whitmoyer and C.H. Sawyer. Anatomy Department and Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Long-term (3-4 wks) ovariectomized (OVX) Sprague-Dawley rats were maintained under standardized lighting and temperature conditions. Unanesthetized, freely-moving animals were bled sequentially at 5-min intervals for 5 hr via indwelling atrial cannulas. From the same plasma samples both LH and FSH secretory patterns were determined by radioimmunoassay. A reconstituted whole blood preparation, consisting of 45-48% rat red blood cells suspended in 5% human plasma protein fraction (Plasmanate, Cutter Laboratories) was infused after each sample was taken to maintain blood volume, hematocrit and osmotic homeostasis. Distinct pulses of LH were observed with a periodicity of about 30 mins, excursions of 200-400 ng/ml/pulse and a half-life (15-20 mins) consistent with that described by other investigators. However, in most animals pulses of FSH were less well defined with a periodicity of approximately 50 mins, excursions of 100-300 ng/ml/pulse and a half-life consistently shorter than the 110 min previously reported by other investigators. Although at times LH and FSH peaks occurred simultaneously, differential secretion patterns were usually observed.

The effects of intracerebroventricular (3V) infusion of catecholamines on the pulsatile secretion of gonadotropins were also tested in these animals. Intraventricular infusion of 0.3 µmoles norepinephrine (NE) in 2 µl over 2 min resulted in rapid and complete inhibition of pulsatile LH secretion, with LH values falling by 85-95%, while FSH levels were affected to a much lesser extent. In most animals plasma FSH values declined after 3V infusion of NE, but the gradual decline did not seem to reflect a mere half-life disappearance of FSH from the plasma. The pulsatile pattern of FSH secretion appeared to continue even though pulses of LH were eliminated. In addition, 3V infusion of 0.3 µmoles clonidine, an α_2-adrenergic agonist, resulted in a biphasic LH response consisting of a transient elevation followed by a prolonged depression in plasma LH but relatively little effect on FSH secretion patterns.

The differences in basic pulsatile rhythm as well as in responsiveness to catecholamines suggest that LH and FSH secretion patterns in OVX rats are under differential control mechanisms. These mechanisms may reside at the levels of GnRH neurons in the brain, gonadotropic cells in the hypophysis, and/or catecholamine receptors in the hypothalamus.
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18.47 PROGESTERONE AND 20α-HYDROXYPROGESTERONE STIMULATE THE IN VITRO MEDIOBASAL HYPOTHALAMIC RELEASE OF LHRH. D.D. Rasmussen* and S.S.C. Yen* (SPON: D. Gibbs). UCSD Sch. of Med., Dept. of Reproductive Medicine, La Jolla, CA 92093.

Circulating levels of progesterone (P) and 20α-hydroxyprogesterone (20α-OHP) increase prior to or concomitantly with the preovulatory LH surge. This in vitro superfusion study examined whether these increases in P and/or 20α-OHP may induce or augment the LH surge by stimulating the release of LHRH from the mediobasal hypothalamus (MBH). Adult ovariectomized rats were implanted with silastic capsules containing 17β-estradiol to simulate the estrogen environment of proestrus. After 3 days the rats were decapitated (1000-1030 h) and MBH fragments from 2 rats were placed in each 0.5 ml superfusion chamber, perfused (0.5 ml/10 min) by oxygenated Media 199 (Gibco) containing 0.05% bovine serum albumin. Following a 90 min stabilization period, effluents were collected at 10 min intervals for 250 min. P or 20α-OHP were dissolved in ethylene glycol or ethanol respectively, and then diluted 1:1000 in superfusion media to a final concentration of 10 ng/ml. Experimental chambers were perfused with 5 pulses (10 ng/ml for 10 min) of P or 20α-OHP at 20 min intervals following an initial 1 hour baseline period, while control chambers were infused with control media only. At 220 min 150 µl of a solution of perfusion media containing 125 mM KCl was injected into each chamber to produce a K^+ concentration of 60 mM after dilution. LHRH was determined in effluent fractions by radioimmunoassay. Mean (±SE, n-6) LHRH release (pg/10 min) in response to both P (9.0 ± 0.5) and 20α-OHP (7.9 ± 0.3) treatment was significantly ($p < 0.01$) greater than that of control treatment (5.3 ± 0.4). The first significant ($p < 0.01$) increase of LHRH release in response to the steroid treatments occurred at 120 min, following the second pulse infusion. The response to KCl stimulation was similar for all treatments. These data demonstrate that in vitro administration of P or 20α-OHP stimulates the release of LHRH by MBH tissue from the adult ovariectomized estrogen-primed rat, an established model for proestrus. It is significant that this effect occurs in tissue fragments containing only the MBH, without the preoptic area. These results suggest that the in vivo increase of circulating P or 20α-OHP associated with the initiation of the preovulatory LH surge may play a role in regulating the timing or amplitude of this surge by directly stimulating the release of LHRH from the MBH. (Supported by NIH grant HD 07203).

18.48 THE ROLE OF SEROTONIN IN THE STEROID INDUCED RELEASE OF PITUITARY LUTEINIZING HORMONE IN OVARIECTOMIZED RATS. S. Iyengar* and J. Rabii (SPON: C. Page). Dept. of Biological Sciences and the Bureau of Biological Research, Rutgers University, Piscataway, NJ 08854.

The role of brain serotonin in the regulation of luteinizing hormone (LH) secretion has become the focus of attention over the last few years. An unequivocal role, however, has not as yet emerged for this neurotransmitter in the hypothalamic control of LH secretion. We have used the estrogen/progesterone induced LH release paradigm as a model for a neuropharmacological approach to this question. Bilaterally ovariectomized adult female rats of the Sprague- Dawley strain were used 2 weeks after castration. On day 1 of the experiment, each rat was injected with an 8 µg dose of estradiol benzoate. This was followed by an injection of progesterone (1.5 mg) on day 3 of the experiment. Blood samples were obtained by venipuncture in the morning of days 1-4 as well as in the afternoon of the 3d day. This steroid injection schedule results in a plasma LH peak 6 hours after the progesterone injection. Treatment of rats with p-chlorophenylalanine (PCPA; 250 mg/kg), the inhibitor of serotonin biosynthesis, in the morning of day 1, led to complete inhibition of the LH surge. At the dose used in this study, 5-hydroxytryptophan (100 mg/kg), the immediate precursor of serotonin, when given at the time of progesterone administration, significantly elevated the LH levels in PCPA pretreated animals, but did not re-establish the expected peak. Similar observations were made with two serotonin receptor agonists, quipazine (30 mg/kg) and N-N-dimethyl-5-methoxy-tryptamine (10 mg/kg). When the serotonin receptor antagonist cyproheptadine (10 mg/kg) was injected at the time of progesterone administration, it was effective in blocking the expected LH surge. Similar results were obtained with two other serotonin receptor blockers. Cinanserin (5 mg/kg) and 2-chloro-2-[3-(dimethylamino)propyl]thio-cinnamanilide-HCl (SQ-10,631; 10 mg/kg) were both effective in inhibiting the steroid induced LH surge. Methysergide (2.5 mg/kg), another presumed blocker of serotonin receptors, on the other hand, was only able to reduce the magnitude rather than block the expected LH surge. We conclude from these observations that serotonin has a stimulatory role in the central nervous system regulation of LH secretion. Supported by funds from the Bureau of Biological Research of Rutgers University.

18.49 CORRELATION BETWEEN INCREASED HYPOTHALAMIC MULTI-UNIT ACTIVITY AND ENDOGENOUS LUTEINIZING HORMONE PULSES IN THE OVARIECTOMIZED RHESUS MONKEY. T. Uemura*, T. Akema*, R. Wilson, J. Kesner* and E. Knobil*. Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

In the rhesus monkey, as in all mammals studied to date, the secretion of pituitary gonadotropic hormones occurs in pulsatile fashion. Each pulse is thought to be the consequence of a packet of hypothalamic LHRH reaching the pituitary by way of its portal circulation. We have recently reported the results of preliminary experiments which demonstrated increases in hypothalamic multi-unit activity (MUA) that corresponded with plasma LH pulses. The present experiments were designed to extend these observations and, to develop techniques for reproducibly recording neural activity associated with LH release.

Using ventriculography and roentgenographic guidance for improved precision in electrode placement, single recording electrodes were placed acutely or multielectrode arrays were implanted chronically at various sites within the medio-basal hypotnalamus (MBH) of 29 ovariectomized rhesus monkeys. For each animal, MUA and LH secretion were analyzed during repeated 8 hr recording sessions. To avoid alterations in hypothalamic activity and LH release coincident with periodic arousal from anesthesia, the animals were maintained on continuous thiopental infusion with EEG monitoring.

In 4 monkeys we recorded MUA increases which were selectively associated with plasma LH pulses. These MUA increases began abruptly just prior to initiation of the LH rise, lasted 30-50 min corresponding to the rising phase of the LH pulses, and terminated near the time of the LH peak. The reproducibility of this association was exemplified in one monkey bearing chronic electrodes in which the MUA-LH coupling was maintained through 5 recording sessions over 8 weeks. Histological analysis in 2 monkeys indicated that electrodes recording MUA-LH correlations were selectively located in the arcuate nucleus region and in the subjacent median eminence. These results confirm our earlier reports (Dufy, J. Physiol. Paris 75:105, 1979; Knobil, Biol. Reprod. 24:44, 1981) of a temporal correlation between activation of neural elements in the arcuate area and the pulsatile release of LH. Additionally, they demonstrate that this coupling can be electrophysiologically monitored over the extended time periods required for analysis of hormonal and neural factors that may modulate this neuroendocrine control system.

18.50 RAPID AND DELAYED CHANGES IN PULSATILE LH RELEASE FOLLOWING OVARIECTOMY IN THE RAT. Robert E. Leipheimer* and Robert V. Gallo* (SPON: Marianne Steele). Department of Physiology, University of California, San Francisco, School of Medicine, San Francisco, CA 94143.

LH release is pulsatile during periods of low level LH secretion in the rat estrous cycle (Gallo, Biol. of Reprod. 24: 771, 1981). The purpose of the present study was to determine the time course of the changes - both rapid and delayed - in LH pulse amplitude and frequency that occur after ovariectomy in the rat. Animals which showed at least two consecutive 4 day estrous cycles were used in these experiments. Unanesthetized rats with jugular cannulae were bled continuously the morning of diestrus 1 (0900-1200 h) or at various times following ovariectomy. Blood samples were taken at 5 to 10 minute intervals, and each rat was only bled once. Ovariectomies were done the morning of diestrus 1 (0830-0900 h), and rats were subsequently bled for a 3 h time period for analysis of pulsatile LH release. Intact diestrous 1 rats demonstrated pulsatile LH secretion with mean blood LH levels of 18 ng/ml whole blood. Following ovariectomy, blood LH levels did not increase by either 10 or 17 h. However, by 1 day post ovariectomy blood LH levels increased to 59 ng/ml whole blood due to an increase in both LH pulse amplitude and frequency. Between 1 and 2 days post ovariectomy blood LH levels remained stable, although some changes occurred in the LH pulse generating mechanism; pulse amplitude increased while frequency decreased, accounting for no net change in mean blood LH levels. Between 2 and 8 days post ovariectomy, a 2½ fold increase in blood LH levels occurred, values reaching 159 ng/ml whole blood. While no further change in LH pulse amplitude occurred during this time, pulse frequency accelerated thereby causing this increase in blood LH levels. Lastly, between 8 days and 3 weeks post ovariectomy an additional 3 fold increase in blood LH levels occurred. However, during this time period LH pulse frequency remained stable but LH pulse amplitude increased. Therefore, these studies demonstrate that: 1) blood LH levels increase rapidly within one day post ovariectomy due to increases in both LH pulse amplitude and frequency; 2) by 8 days following ovariectomy LH pulse frequency reaches a rate that is maintained through 3 weeks, while LH pulse amplitude continues to increase during this time period. Supported by NIH grants HD05577 and AM07265.

18.51 FACILITATORY ROLE OF α-ADRENERGIC MECHANISM IN PULSATILE LH RELEASE IN OVARIECTOMIZED FEMALE GUINEA PIGS. J.A. Keller-Halbe* and Ei Terasawa (SPON. J.W. Kemnitz), Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI 53715-1299

Control mechanisms of the pulsatile release of LH, presumably the pulsatile release of LHRH, in ovariectomized (OVX) animals have not been clarified. Previously, we have reported on the dramatic inhibition by opiate neurons and indirect facilitation by serotonergic neurons on the pulsatile release of LH in the OVX guinea pig (Endocrinology suppl. 108: 290, 1981). The present experiment was performed to further investigate the control mechanism of pulsatile LH release: specifically the possible involvement of catecholamines was tested using antagonists and agonists of α_1 and α_2 adrenergic receptors and dopamine receptors. In order to obtain frequent blood samples from unrestrained and unanesthetized animals, a catheter was implanted in the jugular vein of long-term OVX female guinea pigs (n=33). A day later a 0.5 ml blood sample every 5 min was obtained for the 1 h control period and continued for 3-3.5 h after drugs or vehicles were injected. Plasma LH was established by RIA.

The pulsatile rhythm of LH established in control experiments with and without vehicles, consisted of a pulse duration of 27.0 ±1.4 min, and a pulse amplitude of 621±60 ng/ml at the level of 898±57 ng/ml. Corynanthine (4 mg/kg, ip), an α_1 receptor blocker significantly decreased the mean LH level ($p < 0.005$) and prolonged the pulse duration (48±14 min) 30 min after the drug injection. Prazosin (4 mg/kg, ip), another α_1 receptor blocker, completely suppressed the pulsatile rhythm and the mean LH level 30 min after the drug injection, which was retained for the remaining sampling period ($p < 0.025$). Yohimbine (2-4 mg/kg, ip) an α_2 antagonist, also substantially suppressed the pulsatile rhythm and decreased the mean LH level ($p << 0.001$) 15 min after the drug injection for the remaining sampling period. In contrast, clonidine (0.3 mg/kg, sc) an α_2 agonist, though inducing a slight suppression in LH levels around 20 min, induced a significant increase in mean LH levels and pulse amplitude at 75 min after drug injection, which continued to increase throughout the remaining sampling period ($p < 0.005$). Both haldol (0.5 mg/kg, im), a dopamine antagonist, and bromocryptine (1, 2 and 5 mg/kg, im), a dopamine agonist, did not significantly alter the pulsatile rhythm.

Therefore, it seems that stimulation of both α_1 and α_2-adrenergic receptors facilitates pulsatile release of LH, presumably by interacting with LHRH neurons, while dopamine does not seem to directly affect the release of LH. (Supported by NIH grants RR00167, HD11355, HD15433.)

18.52 WHICH OF THE FOLLOWING CONTROLS CYCLIC RELEASE OF LH IN BABOONS: HYPOTHALAMIC LHRH OR ESTROGEN? N. Hagino, J.W. Lee*, and A. Arimura*. Dept. of Anatomy, Univ. TX Health Sci. Ctr. at San Antonio, TX 78284 and Labs. for Molecular Neuroendrinol. and Diabetes, Depts. of Med. and Anat., Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Previous studies in our laboratory demonstrated that neural signals having 24 hr. periodicity which are located in the forebrain-preoptico region and brain stem travel the diffused forebrain-preoptico tuberal paths, medial forebrain bundle-tuberal paths, and brain stem-tuberal paths to reach hypothalamic LHRH neurons. Circulating estrogen modulates not only neural signals which control hypothalamic LHRH release and the effect of LHRH on LH release, but also potentiates the effect of progesterone on neural signals which control LH release in baboons. However, the physiological role of hypothalamic LHRH in the regulation of cyclic release of LH is not clearly defined.

In this experiment, 6 normally cycling female baboons (papio cynocephalus, 5-7 yrs. old) were injected with anti-LHRH serum to reveal the physiological role of LHRH in cyclic release of LH. Normal circulating levels of estradiol (E_2), LH and progesterone were determined by RIA during the entire period of menstrual cycle preceding the following experiment: In 3 baboons, 10 ml of normal sheep serum was injected through femoral vein under ketamine sedation on a day of peak concentration of E_2 (serum levels of E_2 were determined daily) and injections were given for 5 days to cover the entire period of LH release and ovulation (LH release appears on a day of peak concentration of E_2, reaching a peak on the following day). After 3 months, 10 ml sheep anti-LHRH serum was injected on a day of peak concentration of serum E_2 in these same baboons, and injections were given for 5 days.

Injections of normal sheep serum had no demonstrable effect on serum levels of estradiol and cyclic release of LH, whereas injections of anti-LHRH serum blocked cyclic release of LH without altering estradiol levels. In the second experiment, 10 ml anti-LHRH serum was injected only for 2 days in another 3 baboons; on a day of peak concentration of serum E_2 and the next day. Again, anti-LHRH serum blocked cyclic release of LH. It is suggested that hypothalamic LHRH may play a primary role in the regulation of cyclic release of LH, and that estrogen may modulate this process in baboons. (Supported by NICHD-HD-10071 and HD-14761.)

18.53 PROGESTERONE INDUCED LH RELEASE REQUIRES A TIME INTERVAL AFTER ESTROGEN PRIMING IN FEMALE RHESUS MONKEYS. R.R. Yeoman, N.J. Schultz*, W.E. Bridson* and E. Terasawa, Wisconsin Regional Primate Research Ctr., Univ. of Wisconsin, Madison, WI 53715-1299.

Previously we have reported in rhesus monkeys that a progesterone (P) injection after estradiol benzoate (EB) readily induces an LH surge with a short latency (7h) and a short duration (24h). This LH release with P is quite different from an LH surge (latency 28-48h; duration 48-72h) induced by EB alone. In addition the P-, but not the EB-, induced LH surge, can be blocked by pentobarbital, indicating P stimulates release of LH through the brain, presumably facilitating the release of LHRH. In the present study the interval of EB conditioning for P effectiveness was examined. Long term ovariectomized rhesus monkeys (n=10) received a small estradiol-17β implant 2 wks prior to the experiments. Four experiments were repeatedly performed in which the time of P (2.5 mg) injection was either 0,12,24 or 30h after 10μg EB. Blood samples were obtained through femoral puncture every 3 to 6h. A 4 week minimum rest period was allowed between the experiments. Plasma LH was measured in duplicate with a heterologous double antibody RIA. Administration of P 30h after EB induced a typical LH release with short latency (6.7±0.5h) and duration (22.0±1.5h) in all animals. Similarly, P injection 24h after EB induced an LH release in all animals, which is almost identical to the above (latency; 7.4±0.4h, duration; 25.1±1.9h). The amplitude of the P-induced LH release in both 30 or 24h groups is also similar (P-30h; 65.0±18.7, P-24h; 59.8±24.4 ng/ml). In contrast administration of P 12h or 0h after EB resulted in inducing an LH release in only 50% (P=0.016) and 30% (P=0.0015) of animals, respectively. Furthermore, the amplitude of the LH release in animals responding to P 12h (31.7±20.6 ng/ml) or P 0h (18.7±6.0 ng/ml) was smaller than that of P 30h or P 24h ($P<0.01$). However, the latency and duration of the LH release in animals responding to P 12h (latency; 8.9±0.7, duration; 22.2±2.9h) or P 0h (latency; 7.8±0.2, duration; 15.0±1.7h) were similar to those in animals treated at P 30 and P 24h. These results are interpreted that 1) P injection 24 or 30h after EB reliably induced the LH surge with a short latency and duration and 2) effects of P 0 or 12h after EB are essentially negligible, although some animals responded to P 0 or 12h after EB with low LH amplitude. This is probably due to the effect of an estradiol implant prior to EB injection. It is, therefore, concluded that effects of P in inducing the LH surge in ovariectomized rhesus monkeys requires EB priming more than 12h, but less than 24h, prior to P administration. This interval of estrogen conditioning for progesterone effectiveness is consistent with data reported in rodents on LH release as well as lordosis behavior. (Supported by NIH grants RR-00167, HD11355, HD15433).

18.54 HOW FAST IS PULSATILE LH SECRETION UNLEASHED FOLLOWING CASTRATION? Gary B. Ellis and Claude Desjardins*. Institute of Reproductive Biology, Department of Zoology, The University of Texas at Austin, Austin, TX 78712.

Tonic LH release in the normal male rat is best described as a series of irregular, low-amplitude pulses having a variable frequency. In contrast, long-term castrated rats exhibit regular, high-amplitude, high-frequency LH pulses. In this study, we chart the development of these LH pulses as a function of the time elapsed after removal of the testes. In doing so, we dissect the hypothalamic-pituitary response to castration into neural and endocrine components.

Adult male rats (age >15 wks; wt >450 g) were fitted with an indwelling atrial cannula and loosely tethered to a valve mounted outside the cage. Four days after cannulation, blood samples (0.2 ml) were obtained at frequent intervals (2½-5 min). Withdrawn blood was replaced by a mixture containing 48% rat red blood cells suspended in a human plasma protein fraction. This intensive blood sampling routine was imposed upon castrated rats at 8 time intervals after castration. Blood sampling began at hour 0 (castration occurring during blood sampling); at hours 19, 43, 62, and 120; and on days 21, 80, and 140. At each time interval, 2-4 rats were sampled for 3-7 hours.

The results (values are mean ± SE):

Time after castration	LH pulse peak (ng LH /ml)	Nadir to peak (ng LH /ml)	Interpulse interval (minutes)
0- 7 hr	23.3 ± 2.8	15.9 ± 1.5	161.7 ± 53.3
19- 23 hr	76.5 ± 3.6	37.6 ± 2.6	27.5 ± 2.5
43- 47 hr	133.5 ± 2.2	62.1 ± 3.9	23.2 ± 1.0
62- 65 hr	121.0 ± 5.9	58.1 ± 4.3	29.2 ± 5.9
120-124 hr	145.7 ± 2.8	74.0 ± 4.8	24.4 ± 1.5
21 days	364.2 ± 9.1	127.9 ± 7.8	18.8 ± 1.3
80 days	638.4 ± 18.4	310.8 ± 12.7	21.1 ± 1.0
140 days	792.2 ± 16.1	378.9 ± 30.5	26.3 ± 1.9

The pattern of LH release through 7 hours after castration closely resembles that observed in intact male rats. From 19 hours through 140 days post-castration, the amplitude of LH pulses rises steadily; peak LH levels increase and the pulses grow increasingly pronounced. This graded elevation in LH pulse amplitude is likely a reflection of the remodeling of pituitary gonadotropes that occurs following removal of the testes.

In contrast to amplitude, the frequency of pulsatile LH release is remarkably static from as early as 19 hours post-castration through 140 days. The sudden appearance (<1 day after castration) and uniform frequency of high-frequency LH pulses suggests that in the normal rat an intrinsic, ~25-minute neural periodicity exists and is hidden by testicular influences. [NIH HD-13470]

18.55 LONG-TERM GONADECTOMY ELIMINATES THE EFFECTS OF OPIATES ON LH SECRETION IN THE RAT. R. Bhanot and M. Wilkinson. Depts. of Physiol. Biophys. & Obstet. Gynecol., Dalhousie Univ., Halifax, Canada.

Gonadal steroids have powerful negative feedback effects on LH secretion. Part of this influence is exerted via the hypothalamus, probably through involvement of endogenous opiates. We now show that the inhibitory effects of the opiate peptide FK 33-824 (FK) on LH secretion, and conversely the stimulatory influence of naloxone, are dependent upon recent exposure to gonadal steroids.

Female Sprague Dawley rats were OVX at day 24 and the LH response to naloxone (2.5 mg/kg sc) determined 2,7 and 21 days post-OVX. Naloxone significantly increased serum LH levels (ng/ml; 15 min) from 400±50 (saline) to 780±80 ($p<0.005$) in 2-day OVX rats. This response was attenuated 7 days post-OVX (LH increased from 314±39 to 384±30 $p<0.1$) and was absent in rats OVX for 21 days (LH was 589±47 for controls and 672±75 for the treated group (ns)). These results demonstrate an absence of endogenous opiate suppression of LH release coincident with the disappearance of ovarian negative feedback control.

We subsequently tested the effect of OVX (at day 24) on the ability of FK to inhibit LH release. FK (1 mg/kg, 1.5 hr post-injection) suppressed serum LH by >80% in rats OVX for 2 or 7 days, but was ineffective in animals OVX for 21 days: LH values were 589±47 for controls compared with 700±71 for the FK-treated group (ns). An effect of age was discounted by determination of FK responses in 45-day old rats OVX for 2 days: FK reduced LH levels to 32±13 compared with 125±19 for saline controls ($p<0.005$). Similar results were obtained in males. We therefore determined the effect of testosterone propionate (TP) (1.2 mg/kg/day; x 2 days) on the response to FK in long-term castrates (20 days). FK failed to affect LH secretion in oil-treated controls: serum LH in the oil + FK group was 104±9% of control values. In contrast FK reduced LH levels to 53±13% in TP-primed animals ($p<0.025$ compared with TP + saline). We interpret these findings to indicate that the hypothalamic opiatergic system is serially involved in mediating the regulatory influence of sex steroids on LH secretion. These responses further suggest that the coupling of the opiate receptor to LH regulatory mechanisms is dependent upon gonadal steroids. However, since we have been unable to show any influence of steroids or gonadectomy on hypothalamic opiate binding sites (Wilkinson et al., in 'Steroid Hormone Regulation of the Brain', Eds. Fuxe et al., Pergamon 1981) we conclude that the reversible alteration in opiate involvement in LH release involves another neurotransmitter system. (Supported by Canadian MRC grant to MW).

18.56 MULTIPLE COMPONENTS OF TRH-LIKE IMMUNOREACTIVITY IN RAT HYPOPHYSIAL PORTAL BLOOD. W.J. Sheward*, A.J. Harmar* and G. Fink (SPON: H.W. Kosterlitz). MRC Brain Metabolism Unit, Department of Pharmacology, 1 George Square, Edinburgh, U.K.

In a number of tissues differences between the immunoreactive material (IR) measured by TRH radioimmunoassay and authentic TRH, have been reported (Youngblood et al., 1979). Using reverse phase high performance liquid chromatography (HPLC) combined with a sensitive radioimmunoassay, we have examined the IR material found in rat hypophysial portal blood. Alcoholic extracts of portal blood were evaporated to dryness, reconstituted in buffer and loaded on to the HPLC column. Fractions eluting from the column were dried, redissolved in assay buffer and TRH-like IR determined using ovine anti-TRH serum. Three peaks of immunoreactive material were present in the portal blood, the first of which co-eluted with synthetic pGlu-His-ProNH_2. A single IR peak was obtained from acid or alcoholic extracts of rat hypothalamus and from rat peripheral blood to which synthetic TRH or hypothalamic extracts had been added. Peripheral blood extracts themselves contained little or no immunoreactive material.

Hypophysial portal blood, therefore, contains at least two additional IR substances other than TRH which since they are absent from the hypothalamus are not stored precursors of TRH released into portal blood. However a non-immunoreactive precursor, when released from the hypothalamus, may be degraded to produce intermediates in TRH biosynthesis which do cross react with the antiserum used. Since there is no appreciable cross reaction (<0.1%) between the antiserum and known TRH metabolites and no additional IR peaks were produced after addition of synthetic TRH to peripheral blood, the extra peaks of IR material are unlikely to be TRH metabolites.

(The TRH antiserum was a gift from Dr. H.M. Fraser, MRC Reproductive Biology Unit, Edinburgh).

Youngblood, W.W., Humm, J. and Kizer, J.S. (1979) Brain Research. 163 101-110.

18.57 SOMATOSTATIN INHIBITION OF GROWTH HORMONE RELEASE IS BLOCKED BY PERTUSSIS TOXIN. M. Cronin, A. Rogol*, L. Dabney*, G. Myers*, and E. Hewlett*. Depts. of Physiology & Internal Medicine, Univ. of Virginia Sch. Med., Charlottesville, Va. 22908.

A protein toxin from Bordetella pertussis induces many biological effects including the annulment of dopamine agonist inhibition of prolactin release in vitro (Cronin et al, 1982 Endocrine Society Meeting, abstract 857). To determine whether other inhibitory hormones acting at the anterior pituitary can be influenced by this pertussis toxin (PT), we studied the decrease of basal and stimulated growth hormone (GH) release by somatostatin (33 nM), a physiological GH inhibitory hormone. The PT was prepared from the culture medium of B. pertussis by affinity chromatography on haptoglobin-sepharose (BBA 580:175, 1979). Anterior pituitaries from adult male rats were enzymatically dispersed and plated (500,000 cells/well) for at least 4 days in RPMI-1640 medium supplemented with serum (2.5% fetal calf & 7.5% horse) and antibiotics. The cells were maintained in a humidified chamber with 95% air/5% CO_2 at 37C. Prior to study, cells were exposed to PT for 24 h, a time which allowed maximal toxin effects in other systems. After this treatment, the cells were washed and then exposed to serum-free medium with various combinations of somatostatin and or a GH releasing factor (GRF) (Fed Proc 41: 1488, 1982) for 4 h. Secreted GH was determined by RIA. The PT was able to totally reverse the inhibitory effect of somatostatin on basal GH release without affecting the basal release itself (Table). Furthermore, the PT prevented the somatostatin antagonism of GRF-stimulated GH release (Table) in a dose dependent manner (0.23-230 ng PT/ml). This toxin protein reduces or abolishes inhibitory adenosine, cholinergic, alpha-adrenergic, dopaminergic and now somatostatin receptor effects in a variety of cells. We hypothesize that PT may act at a common, obligatory step involved in the action of these inhibitory receptors.

	Growth Hormone (µg/well; mean ± SEM)			
	− PT	n	+ PT (23 ng/ml)	n
Control	2.76 ± 0.29	5	2.66 ± 0.29	5
Somatostatin	0.94 ± 0.10*	6	2.73 ± 0.31	6
GRF	8.88 ± 0.38**	6	9.25 ± 0.32**	6
GRF + Somatostatin	2.07 ± 0.18	6	7.87 ± 0.48*	6

* different from 7 other groups ($p < 0.05$ to 0.01)
** different from 6 other groups ($p < 0.05$ to 0.01)
except each other: statistics by ANOVA.

(Supported by RCDA 1K04N500601, RCDA AM-00153 & AM-22125).

18.58 A COMPARTMENTALIZED ORGAN-CULTURED HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM FOR THE STUDY OF VASOPRESSIN RELEASE. Christine M. Gregg* and Celia D. Sladek. (Spon: E. Hibbard) Dept. of Biology, Pennsylvania State Univ., Univ. Park PA 16802 and Depts. of Anatomy and Neurology, U. Rochester Sch. of Medicine and Dentistry, Rochester, NY 14642.

An organ-cultured compartmentalized hypothalamo-neurohypophysial system (CHNS) has been developed in order to test whether substances affecting vasopressin release act on hypothalamus (HT) or directly on posterior pituitary (PP), or both. Hypothalamic explants containing supraoptic nuclei, median eminence, and PP were removed from decapitated male Sprague-Dawley rats (175-200g). Hypothalamus and pituitary were maintained in organ culture in separate compartments and the intact infundibular stalk passed through a small opening in the fluid-tight barrier which separated the 2 sides. The only communication between the compartments is neuronal. Thus, either side may be selectively stimulated and the locus of action on VP release determined. VP was measured by radioimmunoassay.

The amount of VP released from PP on 4 successive days was 2007±23, 1156±83, 1058±81, and 451±38 pg/24 hr. Respective values for HT side were 175±16, 96±13, 149±17, and 118±11 pg/24 hr. Values do not include a correction for simultaneous degradation which can approach 100pg/hr on PP side.

Experiments were done on days 2 & 3. PP VP release was measured during a control hour and again during a subsequent test hour during which a stimulus was administered on HT side. VP release during the test hour is expressed as a % of the control hour release.

Stimulus to HT side	none	↓ Osm 6 mOsm	↓ Osm 15 mOsm	↑ Osm 15 mOsm	+ Ach 10^{-5} M
PP VP release (% of control)	85%±13%	46%±6%	29%±7%	708%±342%	140±11%
(n)	(6) *p=.29	(5) p<.001	(6) p<.001	(5) p=.07	(5) p<.025

osm=osmolality Ach=acetylcholine *paired t-test

Basal PP release rates during the control hour for these experiments was 194±43 pg/hr.

Four day old explants stained for VP neurophysin showed immunoreactivity in supraoptic neurons, fibrous zone of median eminence, and PP.

The organ-cultured CHNS responds appropriately to known stimuli for VP release and should prove to be another useful tool for the study of VP release. Supported by NIH grant NS 17300.

18.59 CHARACTERIZATION AND LOCALISATION OF NEUROTENSIN IN THE RAT ANTERIOR PITUITARY GLAND. M. Goedert, P.C. Emson and S. Lightman (SPON: M. Hanley). MRC Neurochemical Pharmacology Unit, MRC Centre, Hills Road, Cambridge, England.

Using both amino- and carboxy-terminal specific neurotensin antisera we detected neurotensin-like immunoreactivity (NTLI) in the rat anterior pituitary (80 pmol/g). This NTLI could not be distinguished from synthetic neurotensin on gel or high-performance liquid chromatography. The NTLI is localised to a specific population of cells in the rat anterior pituitary from which it can be released by depolarization. The neurotensin content of the anterior pituitary in vivo was dramatically reduced by propylthiouracil (PTU) treatment. Within one week of PTU treatment the neurotensin content of the anterior pituitary decreased by more than 90%. The effect of PTU could be reversed or prevented by treatment with L-tri-iodothyronine (T_3) suggesting that the fall in neurotensin content was attributable to a fall in thyroid hormone induced by PTU treatment. In agreement with this suggestion thyrotropin releasing hormone (TRH) treatment (Alzet minipump 50µg/kg/day) also produced a 90% decrease in neurotensin content. This data suggests a functional relationship between pituitary neurotensin and the thyroid although the nature of this relationship is unclear at present.

18.60 EFFECTS OF URETHANE ON PITUITARY-ADRENAL FUNCTION. W.N. Hamstra*, S. Orr*, D. Doray*, and J.D. Dunn. Dept. of Anatomy, Oral Roberts University., Sch. of Med., Tulsa, OK 74171.

Urethane anesthesia is frequently used in studies concerned with testing neuronal responsiveness to a variety of stimuli including hormonal administration. However, little is known about its mechanisms of action or its effect on hormonal systems, particularly pituitary-adrenal function. Since both ACTH and adrenal corticosteroids have marked effects on neural activity of various CNS sites, we undertook a systematic evaluation of the effect of urethane on pituitary-adrenal function.

Urethane (1.35 gm/Kg, i.p.) or saline was administered to variously treated adult female rats and timed blood samples were taken from an exposed jugular vein or via rapid decapitation. Plasma levels of corticosterone (Cpd B), an index of pituitary-adrenal activity, were determined fluorometrically.
Urethane produced a rapid and sustained increase in Cpd B levels both in the AM and PM. Initial corticosterone levels of non-injected control rats showed marked AM-PM differences, but by 10 min post-injection morning Cpd B levels were increased such that AM-PM differences were not observed. By 30 min post-injection, afternoon plasma Cpd B levels had increased significantly but AM-PM differences were still abolished. AM and PM Cpd B levels remained elevated for at least 4 hrs. Saline injected controls showed the expected response to stress; plasma Cpd B levels were significantly increased at 10 min but were back to baseline by 30 min. Dexamethasone (100 µg/Kg, s.c.) markedly suppressed both AM and PM urethane stimulated Cpd B levels, but diurnal differences in dexamethasone suppression were noted; increased dexamethasone (250 µg/Kg) administration abolished the diurnal difference. Neither AM or PM infusions (10 min) of exogenous corticosterone (8 µg/ml/min, i.v.) significantly altered plasma Cpd B levels. Hypophysectomy abolished the urethane induced adrenocortical activity, and plasma Cpd B levels of non-anesthetized, hypophysectomized ACTH-primed and injected rats were not different from those similarly treated and anesthetized with urethane. Urethane-stimulated plasma Cpd B levels were significantly reduced 15 min after intravascular infusion of atropine (0.8 mg), but urethane-induced increases in Cpd B were not affected by hypothalamic deafferentation.

Collectively, these data indicate that urethane evokes a sustained increase in pituitary-adrenal activity, that the increased activity is dexamethasone and atropine sensitive and that the site of action is the hypothalamo-hypophyseal complex. Extrahypothalamic CNS sites may be influenced by urethane anesthesia but urethane-induced increases in pituitary-adrenal activity do not necessitate extrahypothalamic neural influences.

18.61 SECRETAGOGUE-INDUCED THYROTROPIN RELEASE IS DECREASED BY PENFLURIDOL: A POSSIBLE ROLE FOR CALMODULIN. A.M. Judd,* G. Schettini* and R.M. MacLeod*. (SPON: F.E. Dreifuss). Dept. of Internal Medicine, Univ. of Virginia School of Medicine, Charlottesville, VA 22908.

We have studied the effects of penfluridol (PF), a potent neuroleptic, on basal and secretagogue-induced thyrotropin (TSH) release. Stimulus secretion coupling in various cells is known to involve calcium-dependent mechanisms, and calcium is thought to have its effect via binding to and thereby activating the protein calmodulin (CM). Several pharmacological agents, including neuroleptics, in addition to their effects on neurotransmitter receptors, have been reported to bind to and inhibit CM. To determine the effect of PF on basal TSH release, female rat hemipituitaries were incubated in Medium 199 under a 95% O_2, 5% CO_2 atmosphere for 5 hrs at 37° and the TSH content of the gland and medium determined by radioimmunoassay. PF (100 μM) had no effect on unstimulated total, glandular or medium TSH content. The inability of PF to modify basal TSH release may be explained by the observation that basal TSH release seems to be a Ca^{2+}-independent process, whereas the stimulus-related release of TSH seems to be a Ca^{2+}-dependent process. To investigate the effect of PF on secretagogue-induced TSH secretion, a second series of experiments was carried out in a manner analogous to the first, except the hemipituitaries were treated with vehicle or PF for the first 2.5 hrs and then the medium was removed and the pituitaries incubated for 30 min in fresh medium containing secretagogue and either vehicle or PF. PF (0.1 - 10 μM) had no effect on basal TSH release but decreased in a dose-related manner the release of TSH due to either 70 nM thyrotropin releasing hormone (TRH), 50 mM K+, 10 μM calcium ionophore A-23187, 3 mM dibutyryl cAMP, or 5 mM theophylline. Since all of these secretagogues affect TSH secretion via Ca^{2+}-dependent mechanisms, the PF effect may be due to inactivation of CM.

If the PF effect occurs via inactivating CM, then it should be reversible. To test this hypothesis, enzymatic dispersed pituitaries were intermittently perifused with Medium 199 containing vehicle or PF (1 μM) and the release of TSH due to 70 nM TRH determined. TRH induced a marked increase in TSH release (250%), which was completely blocked by PF. The responsiveness of the cells to TRH returned subsequent to withdrawal of the PF. Further exposures to PF blocked TRH-induced TSH release, with the recovery of the TSH response to TRH following the withdrawal of each PF perifusion.

In conclusion, although PF does not affect basal TSH secretion, it decreases TSH release caused by a number of secretagogues. In the case of TRH, the PF effect is reversible. These observations are consistent with the hypothesis that PF inactivates the calcium-CM system, and suggests that CM may play a role in the secretagogue-induced secretion of TSH. (Supported in part by USPHS Grant CA-07535 from the National Cancer Institute)

18.62 NEONATAL ADRENAL TRANSPLANTATION ALTERS NORMAL PITUITARY-ADRENOCORTICAL RELATIONSHIPS. W.C. Engeland*, C. Wilke* and S. Feeley* (SPON: C. Allen-Rowlands). Div. of Biol. Med., Brown Univ. and Rhode Island Hosp., Providence, RI 02902.

To define the effect of disruption of adrenal nerves on the development of the rhythms of plasma corticosterone (B) and of plasma ACTH, adrenals from neonatal (day 1) rats were transplanted unilaterally into the anterior chamber of the eye (NTE) of adult bilaterally adrenalectomized recipients. Groups of rats were sacrificed by decapitation in the AM and in the PM at 1,3,5, 7 and 9 weeks after transplantation. Control rats, littermates to adrenal donors, were sacrificed at 1,3,5,7 and 9 weeks of age. Plasma B and ACTH concentration were determined by RIA. Plasma B in the PM was significantly greater ($p<0.05$) than in the AM at 5,7 and 9 weeks in both the NTE and intact groups. In the AM and in the PM, there was no difference ($p>0.05$) in plasma B between intact and NTE rats at any time. Plasma ACTH in the AM and the PM was not different ($p>0.05$) at any time in the NTE and intact groups. However, plasma ACTH in NTE rats was significantly greater ($p<0.025$) than in intact rats at all times. To determine whether or not differences in plasma ACTH following transplantation were related to the site of transplantation or to the age of the transplanted adrenal, plasma ACTH and B were determined in the PM in groups of adult rats at 5 weeks after either NTE, unilateral neonatal adrenal transplantation to the kidney capsules (NTK), unilateral adult adrenal transplantation to the kidney capsule (ATK), or unilateral adrenalectomy and sham transplantation to the kidney capsule (STK). There was no difference in adrenal weight ($p>0.1$) or in plasma B ($p>0.1$) between groups. Whereas plasma ACTH in STK and in ATK groups was not different ($p>0.5$), plasma ACTH in both NTE and NTK groups was significantly greater ($p<0.025$) than that in the STK and ATK groups. Thus, transplantation of the neonatal adrenal into an adult recipient results in elevated plasma ACTH and normal rhythmicity in plasma B. These findings suggest that adrenal transplantation neonatally prevents the maturation of a neural or humoral mechanism that is necessary for the normal development of the pituitary-adrenocortical system. (Supported in part by BRSG 7655 and NIH AM 26831).

19.1 SEXUAL DIFFERENTIATION OF BRAIN FUNCTION : THE ROLE OF GONADAL HORMONES IN ELECTROCONVULSIVE SHOCK,
C-A. Hardy*, Z. S. Dolinsky, P. J. Donovick, and R. G. Burright* (SPON: E. Shaskan, Dept of Psychiatry, UCONN, Farmington). Dept. of Psychology, SUNY-Binghamton, Binghamton, N.Y. 13901.

The duration and severity of transcorneally-induced electroshock seizures of 93 HET male and female mice was assessed at 63 days of age following removal of the gonads or sham-operations at either 21 days of age or 42 days of age. An additional group of 24, 60-65 day old intact non-surgical female mice was tested exclusively to determine the relationship between the phases of the estrous cycle and transcorneally-induced seizure susceptibility. The duration and severity of behavioral seizures was similar for intact males and intact females. However, intact females classified as being in estrous or proestrous were more likely to seize following electroconvulsive shock than those intact females classified as in metestrous or diestrous. This finding suggests that when circulating levels of estrogens are high, young adult female mice have a heightened susceptibility to electroshock seizures, presumably due to the central effects of estrogens. However, gonadectomized animals, on the average, tended to have somewhat longer behavioral seizures than intact animals, suggesting that heightened susceptibility to electroshock seizures is not simply a function of the level of circulating estrogens. Also, mice operated on at 21 days of age tended toward longer behavioral seizures than those mice operated on at 42 days of age.

All mice were sacrificed following testing and brain, pituitary, liver, adrenals, kidneys, spleen, and ovaries, or seminal vesicles and testes were removed wherever possible, and weighed. Marked effects of gonadectomy were noted for spleen, kidney, seminal vesicle, brain, and adrenal weights. Furthermore, intact male and female organ weight differences were noted, as well as organ weight changes for each of the four classified phases of estrous in the intact females. In conclusion, while gonadectomy produced pronounced peripheral organ weight changes, it is unclear what effect gonadectomy has on brain seizure mechanisms, and further investigation is warranted.

19.2 ADRENALECTOMY AND METOPIRONE DISRUPT ACQUISITION OF ACTIVE AVOIDANCE BEHAVIOUR.
R. J. Bialik*, B. A. Pappas and D. C. S. Roberts (SPON: T. Picton). Unit for Behavioural Medicine and Pharmacology, Dept. of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Investigations into the role that adrenal steroids play in active avoidance learning have yielded conflicting results. These studies have usually employed bilateral adrenalectomy to reduce corticosterone levels in plasma. One problem encountered when using adrenalectomized rats is that the corticosterone levels are not completely reduced, often remaining at 20 to 30% of controls. Another is that these rats require a week or longer to recover following surgery. Testing this long after surgery allows for compensatory changes in steroid uptake in the brain or increased extra adrenal synthesis of corticosterone, thereby complicating interpretation of the results.

To test the effect of acute corticosterone depletion on avoidance behaviour the corticosterone synthesis inhibitor Metopirone was used here. Injection of Metopirone (50 mg/kg ip) resulted in plasma levels of corticosterone which were reduced to 40% of control values two hours after injection. Two hours following Metopirone injection, rats showed a deficit in the acquisition of a shuttle-box avoidance response like that seen three weeks following adrenalectomy. Administration of Metopirone to adrenalectomized rats also caused a further avoidance deficit and corticosterone depletion. This Metopirone-induced avoidance deficit was dose dependent over the range of 0, 10, 25 and 50 mg/kg. The deficit could be attenuated but not completely reversed by subcutaneous implantation of pellets containing corticosterone or dexamethasone. The seventh daily injection of Metopirone (50 mg/kg ip) reduced avoidance performance and corticosterone levels similar to that of a single injection of the drug, thus indicating an absence of tolerance to its behavioural and endocrinological effects.

Metopirone is used clinically to test for anterior pituitary dysfunction. That there is a behavioural impairment after acute and chronic Metopirone in the rat suggests that the behavioural effects of this drug in humans should be explored. (Supported by MRC and NSERC)

19.3 PSYCHONEUROENDOCRINE EFFECTS OF SEPTAL AND STRIATAL DAMAGE IN THE RAT.
T.R. King* and D.M. Nance (SPON: J.A. Armour). Department of Anatomy, Dalhousie University, Halifax, N.S., B3H 4H7.

With respect to female sexual behavior, electrolytic septal lesions increase sensitivity to sex steroids. This facilitation in lordosis behavior is associated with changes in brain dopomine (DA) and GABA (Brain Res. Bull. 2:341, 1977). Here we report the effects of neurotoxic lesions in the septum and striatum induced by 6-hydroxydopamine (6-OHDA) or kainic acid (KA) on body weight (BWt), ovarian compensatory hypertrophy (OCH) and lordotic behavior. The 6-OHDA was injected bilaterally into the lateral septum (8 µg in 1.0 µl) and striatum (16.0 µg in 2.0 µl). Likewise, KA was injected into the septum (0.5 µg in 1.0 µl) and striatum (1.0 µg in 2.0 µl). Electrolytic (DC) septal lesion rats (2mA/20") as well as vehicle and sham operated controls were included. Effectiveness of 6-OHDA injections was subsequently verified by measuring brain DA levels using HPLC and electrochemical detection. Relative to controls, septal injections of 6-OHDA selectively reduced septal levels of DA by 60%. Striatal injections of 6-OHDA reduced striatal levels of DA by 77%, septal levels by 30% and had no effect on DA levels in the accumbens. Brain histologies indicated that the DC lesions bilaterally destroyed the lateral and occasionally medial septum. The KA septal lesions reduced the overall size of the septal area (primarily lateral region), but otherwise the septum appeared remarkably normal at the light microscopic level. However, there was extensive neuronal cell loss in hippocampal areas CA3 and CA4. KA-striatal injections were found to be lethal with only 1 survivor. Behavioral and endocrine effects of these various lesions were, relative to controls; 1) KA in the septum increased and 6-OHDA in the striatum decreased BWt, 2) 6-OHDA in the striatum reduced ovarian weight and KA in the septum increased OCH, 3) DC septal lesions increased whereas KA septal lesions decreased female sexual behavior. Also, 6-OHDA septal lesions reduced lordotic behavior. These results suggest that decreases in brain DA associated with DC septal lesions are not causally related to the increased female sexual behavior. The opposite effects of DC and KA septal lesions on lordotic behavior were unexpected and possibly related to the extensive hippocampal damage associated with the KA injections. Likewise, the increase in OCH by the KA septal rats may also be related to the hippocampus, a region proposed to exert inhibitory control over gonadotropin release. Supported by MRC Grants #MA-6807 and MA-6956.

19.4 IMMUNOREACTIVE SOMATOSTATIN LEVELS IN BRAIN REGIONS OF ELECTRICALLY KINDLED VS CHEMICALLY KINDLED RATS.
T. Higuchi*, K.R. Shah*, N. Kato, M. West*, J.A. Wada* and H.G. Friesen*, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, and Health Sciences Center Hospital, The University of British Columbia, Vancouver, B.C.

The kindling phenomenon has become a useful model for studying partial seizures and epileptogenesis. Although there have been many biochemical findings in kindled animals, in many instances these findings reflect not the basic mechanism of kindling but the consequences of kindled seizures. Somatostatin has been shown to induce epileptic-like EEG activity and generalized seizures (GS) in rats.

In the present study, we investigated immunoreactive somatostatin (IRS) levels in brains of rats after electrical and chemical kindling. Electrical kindling: Fourteen Long-Evans male rats (250-300 g) were implanted stereotaxically with electrodes in both amygdala. Stimulation was administered through left amygdala once daily with 1 second trains of 6.0 Hz sine wave at an intensity of 150 µA. Stimulation was stopped after five stable stage 5 (GS) were elicited. It took 9.7 days (average) to develop stage 5. After 2 months of establishment, kindled rats and controls were killed by microwave irradiation and brains dissected into 7 regions (Amygdala, Sensorimotor Cortex, Hippocampus, Piriform Cortex, Entorhinal Cortex, Hypothalamus, Cerebellum). Tissues were extracted in 0.1 N acetic acid and the supernatant was stored at -70°C until IRS assay. Chemical kindling: Sixteen male SD rats (246 ± 11 g) were given subthreshold doses of Lidocaine (60 mg/kg) by I.P. route once daily. Administration of Lidocaine was continued until the onset of G.S. within 5 min. (kindling). In 7 out of 16 rats, the kindling was established after an average of 27 (7-40) Lidocaine injections. After 1-3 weeks of establishment, the same dose of Lidocaine was administered again and the kindling effect was confirmed. One week later the kindled rats (7), non-kindled rats (9) which did not show GS after chronic injection of Lidocaine, and control rats were killed and the brain tissues assayed for IRS in a similar fashion to electrically kindled rats. Results: In electrically kindled rats, a significant increase of IRS was found in Amygdala*** (119% of controls), Entorhinal Cortex* (32%), Piriform Cortex**** (63%), and Sensorimotor Cortex**** (37%). In chemically kindled rats, IRS showed a significant increase in Amygdala** (56% of controls) and Entorhinal + Piriform Cortex* (50%). In non-kindled rats, IRS increased significantly only in Amygdala** (58%). (*P< 0.5, **p< .02, ***p< .01, ****p< .001). The results suggest that somatostatin may be associated with both electrically and chemically induced kindling.

19.5 INDEPENDENT EFFECTS OF ESTRADIOL ON FEEDING AND DRINKING. J.A. Czaja*, P.C. Butera* and T.A. McCaffrey* (SPON: F.R. Brush). Department of Psychological Sciences, Purdue University, West Lafayette, IN 47907.

Six experiments examined the effects of estradiol on feeding and drinking in female guinea pigs. Estradiol treatment was found to reduce water intake independently of its actions on food intake and body weight.

Initial observations indicated that intact female guinea pigs display a cyclic variation in feeding, drinking and body weight across their ovarian (estrous) cycles. Minimum intake and body weight coincided with rupture of the vaginal membrane, the estimated time of ovulation. Compared to the midluteal phase of the cycle, the periovular depression averaged 15.1 ± 1.9% for food intake, 11.3 ± 4.9% for water intake and 1.7 ± 0.2% for body weight. In a second experiment utilizing ovariectomized females, injections of 3 µg estradiol benzoate per day significantly depressed food intake by 24.9 ± 0.4%, water intake by 17.6 ± 4.1% and body weight by 2.0 ± 0.6% compared to oil injections. Neither during the estrous cycle nor following estradiol injections did the ratio of water intake per gram food intake change significantly. This suggested that the reduced drinking might be a consequence of the reduced feeding. To test this hypothesis, food rations were reduced to 30 percent below ad lib levels in experiment #3. This partial reduction in feeding by itself had no significant effect on drinking. In experiment #4, therefore, ovariectomized females were first placed on a food ration 30 percent below ad lib levels and then injected with either 3 µg per day estradiol benzoate or oil. Compared to oil injections, these estradiol injections significantly reduced water intake by 21.0 ± 6.0% while food intake declined insignificantly by only 0.2 ± 0.1%. In these experiments, the reduction in food intake was therefore neither a sufficient nor a necessary condition for the estradiol induced suppression of water intake.

The last two experiments verified that estradiol has independent actions on feeding. The daily water ration was reduced to 30 percent below ad lib levels in experiment #5 with no significant effect on food intake. In the sixth experiment, the water ration of ovariectomized females was first reduced to 30 percent below ad lib levels. Females were then injected with either oil or 3 µg per day estradiol benzoate. With this reduced water ration, the estradiol significantly suppressed food intake by 11.3 ± 4.1% while producing only minimal and insignificant changes of 0.2 ± 0.6% in water intake.

Our findings establish that estradiol can independently influence water intake and food intake in the guinea pig, thereby indicating that estradiol operates through different mechanisms to produce these two effects.

19.6 EFFECTS OF STEROID HORMONES ON THE CNS AND ITS RESPONSIVENESS TO VAGINOCERVICAL STIMULATION, T. O. Allen, Y. Gomita*, and N. T. Adler. Dept. of Psychology, University of Pennsylvania, 3815 Walnut St., Philadelphia, PA 19104.

Stimulation of the female rat's vaginal cervix by the male during coitus (or by copulomimetic stimulation) has several behavioral and physiological consequences, for example potentiation of the lordotic posture, and initiation of twice-daily surges of prolactin necessary for pregnancy. Gonadal hormones facilitate these effects. We have continued to investigate the nature of the central nervous system mediators of copulatory stimulation (Allen et al., Science, 1981, 211:1070). We have expanded our investigation to assess the influence of steroid hormones (estrogen & progesterone) on the brain's response to copulomimetic stimulation. For our study of hormonal mediators, 24 female rats were bilaterally ovariectomized and injected with either a) no exogenous hormones, b) estrogen only, c) progesterone only, or d) sequential estrogen and progesterone. Half of the rats in each group were given vaginocervical stimulation during incorporation of 14C deoxyglucose. The other half did not receive vaginocervical stimulation.

Computerized image-processing of 47 brain nuclei revealed that in females not receiving vaginocervical stimulation estrogen generally increases uptake of 2DG (36/47 structures) and progesterone decreases uptake (39/47 structures). Treatment with estrogen and progesterone produced variable patterns of 2DG uptake across the 47 brain areas analyzed.

In females treated with estrogen and progesterone, vaginocervical stimulation increased uptake of 2DG in 41/47 brain areas. Similarly, in females receiving either progesterone or no hormone, vaginocervical stimulation also produced increased metabolism. However, in females receiving only estrogen, vaginocervical stimulation did not increase brain activity.

There were correspondences between the 2DG map of functional activity in this study and maps of steroid-hormone concentrating cells (e.g. Pfaff & Keiner, J. Comp. Neurol. 151:121). Previous reports (Moguilevsky and Malinow, Amer. J. Physiol. 1964, 206: 855; Schiaffini & Marin, 1975, Excerpta Med. Int. Congr. Ser. 374:123) of metabolic changes in the hypothalamus during the estrous cycle may be related to changing titers of steroid hormones.

19.7 PRENATAL HORMONAL INFLUENCES ON TAIL POSTURE ASYMMETRY IN NEONATAL RATS. G.D. Rosen*, A.S. Berrebi*, D.A. Yutzey, and V.H. Denenberg. Dept. Biobehavioral Sci., U. of Conn., Storrs, CT 06268.

Ross, Glick and Meibach (PNAS, 78: 1958, 1981) demonstrated a sexually dimorphic pattern of asymmetry in the tail posture of neonatal Sprague-Dawley rats. A sexually dimorphic pattern of tail posture has been reported for Purdue-Wistar rats as well (Denenberg et al., Dev. Br. Res., 2: 417, 1982): males and females were biased to the left with females more biased than males. Also, more males had a neutral tail posture than females. The question addressed here was whether exogenous prenatal hormones could influence the asymmetry pattern of tail posture in the rat.

On days 16-21 of gestation, pregnant Purdue-Wistar rats were injected with testosterone proprionate (TP), dihydrotestosterone proprionate (DHTP), sesame oil, or received no injection. At birth, tail posture was assessed and then sex was determined. There was no difference between the oil-injected and the non-injected groups and the data were pooled. The results are summarized in Table 1.

The control animals replicated our previous work with both males and females biased to the left and more males with neutral tail postures than females. The TP-injected females were significantly different from the control females ($p < 0.01$) but were not different from the control males ($p > 0.10$). Females receiving DHTP were no different from control animals ($p > 0.10$) but were significantly different from TP-injected females ($p < 0.02$).

The population pattern of neonatal tail posture asymmetry can be affected by TP but not DHTP injected prenatally. DHTP has no effect on the sexually dimorphic pattern of tail posture asymmetry. DHTP is a non-aromatizable androgen having peripheral effects. The conversion of testosterone to estrogen in the brain has been hypothesized to underlie sexual differentiation in the rat. Thus, the difference between the TP- and the DHTP-injected groups in this study suggests a central mechanism for tail posture asymmetry.

TABLE 1

Number of Animals with Tail Posture
(percentages in parentheses)

	LEFT	NEUTRAL	RIGHT
MALES	92 (55.8)	20 (12.1)	53 (32.1)
FEMALES	116 (66.3)	6 (3.4)	53 (30.3)
FEMALES + TP	17 (37.8)	8 (17.8)	20 (44.4)
FEMALES + DHTP	59 (62.8)	5 (5.3)	30 (31.9)

19.8 THE ABILITY OF PROGESTERONE TO RELEASE LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) IN VITRO FROM IMMATURE FEMALE RAT HYPOTHALAMIC FRAGMENTS IS NOT RELATED TO CYTOPLASMIC PROGESTIN RECEPTOR LEVELS. T. W. Jasper and V. D. Ramirez. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

We have reported that intermittent but not continuous infusion of progesterone (P) stimulates LHRH release in vitro from hypothalamic fragments from ovariectomized, estrogen-primed immature rats (Kim and Ramirez, Endocrinology, 1982, in press). The following study was conducted to examine the possible relationship between cytoplasmic progestin receptor (PRc) levels and the ability of P to stimulate LHRH release in vitro from the immature female rat hypothalamus.

Immature 28-day-old female rats were ovariectomized and implanted sc with silastic capsules containing estradiol-17β (10 mm length, 1.6 mm width id, 235 µg/ml oil). (This estrogen dose stimulated hypothalamic-preoptic area PRc levels nearly 10-fold versus ovariectomized-only immature rats by 30 days of age; 11.17±1.06 versus 1.23±0.74 fmol/mg protein, respectively.) At 30 days of age, the rats were decapitated and the mediobasal hypothalamic-anterior hypothalamic-preoptic area (HPOA) fragments were removed, placed in superfusion medium on ice, and subsequently transferred to superfusion chambers maintained at a constant temperature. At various times during the superfusion, HPOA fragments were removed from the chambers and placed in superfusion medium on ice for the duration of the experiment. After superfusion, the fragments were homogenized in assay buffer and PRc levels were measured in the 45,000 g x 60 min supernatant using ^{3}H-R5020, a synthetic progestin, as the specific ligand.

Incubation of the HPOA fragments at a temperature of 37C resulted in a rapid exponential decrease in PRc levels (the calculated half-life was 6 min). At 30C, the exponential decrease in PRc levels was greatly reduced, and the corresponding half-life of PRc decay was calculated to be 55 min. However, the reported ability of P to release LHRH in vitro from the HPOA fragments was markedly reduced when the units were superfused at 30C versus 37C (see Kim and Ramirez, Endocrinology, 1982, in press). Infusions of norepinephrine, a possible mediator of estrogen stimulation of PRc levels in female rats (Nock et al., Brain Res. 207: 371-396, 1981), were ineffective in preventing the loss of PRc at 37C. The loss of PRc is apparently not the result of nuclear translocation, as extraction of the 45,000 g x 60 min pellet with KCl did not yield detectable PR at the end of the superfusion period.

These data are inconsistent with the hypothesis that the in vitro effect of P to stimulate LHRH release is mediated by a genomic mechanism involving PRc binding, and an alternative mechanism of action for P must be considered.

19.9 MODULATION OF LOCAL CEREBRAL GLUCOSE METABOLISM BY ESTROGEN AND PROGESTERONE IN THE HYPOTHALAMUS OF OVARIECTOMIZED FEMALE RATS. L. Porrino*, H. Namba*, A. Crane*, J. Jehle*, L. Sokoloff. Laboratory of Cerebral Metabolism, NIMH, Bethesda MD 20205.

The gonadal hormones, estrogen and progesterone, have been shown to have potent influences in the central nervous system, particularly in the hypothalamus. The 2-deoxyglucose autoradiographic method was used to identify the neural circuits upon which these hormones act to regulate sexual behavior in the female rat. Three groups of female Sprague-Dawley rats ovariectomized (OVX) 2-3 weeks prior to study were used: 1) OVX control; 2) OVX implanted subcutaneously with Silastic capsules containing 10% estradiol (E2) for 48 hours; and 3) OVX, implanted as group 2, injected with 500 µg progesterone (P) 4 hours before the start of the experiment. Local cerebral glucose metabolism (LCGU) was studied by the deoxyglucose method with particular emphasis on the nuclei of the hypothalamus. E2 treatment significantly increased LCGU in the anterior, ventromedial, lateral and posterior hypothalamic areas when compared to OVX controls. No significant changes were seen in any other hypothalamic region. In contrast, in animals receiving E2 + P treatment, LCGU was reduced in the lateral preoptic, magnocellular portion of the preoptic area and suprachiasmatic nucleus, as well as in the anterior hypothalamus in comparison to metabolism in either OVX-control or E2-treated rats. P injections did not alter LCGU's in the ventromedial, lateral or posterior nuclei, areas which showed increases following E2 alone.

These data demonstrate an anatomical separation of the effects of gonadal steroids in the hypothalamus. E2 facilitates neural activity in the mid and posterior portions of the hypothalamus, while E2 in combination with P suppresses activity in the anterior-preoptic region. The two distinct patterns of alteration of LCGU in the anterior and posterior regions of the hypothalamus may reflect differential involvement in feminine sexual behavior.

19.10 CONTROL OF FEMININE SEXUAL BEHAVIOR WITH INTRAVENTRICULAR INFUSION OF CHOLINERGIC AGENTS. P.J. Barr*, G.P. Dohanich, and L.G. Clemens. Hormones and Behavior Laboratory, Michigan State Univ., East Lansing, MI 48823.

Sexual behavior in the female rat is regulated by the ovarian hormones, estrogen and progesterone. Evidence indicates that this behavior, characterized by the postural lordosis response to a mount by a male, may be mediated within the brain by cholinergic mechanisms. The experiments reported further analyze cholinergic influences on lordosis.

Ovariectomized female rats were implanted with bilateral cannulae in the lateral ventricles. In the first experiment, females were primed intramuscularly with 0.13 µg estradiol benzoate (EB) for 3 days prior to intraventricular infusion of carbamylcholine chloride (carbachol), a cholinergic agonist. This estrogen regimen by itself fails to activate sexual behavior in female rats. A dose-related facilitation of lordosis was observed over the 4 concentrations of carbachol used (0.125, 0.25, 0.5, 1.0 µg/cannula in 0.5 µl volumes). Highest levels of response were observed 15 minutes after intraventricular infusion. This facilitation was completely prevented by systemic pretreatment with 2 mg atropine sulfate, a muscarinic receptor blocker. In a second experiment, ovariectomized females were primed with 0.13 µg EB for 3 days and intraventricularly infused with eserine sulfate, an acetylcholinesterase inhibitor. Facilitation of lordosis occurred in a dose-dependent manner over 3 concentrations (2.5, 5.0, 10.0 µg/cannula in 0.5 µl volumes). Highest responses were observed 15 minutes after infusion. In the third experiment, females were primed with 0.25 µg EB for 3 days and 0.5 mg progesterone on day 4. This estrogen-progesterone regimen by itself activated a high level of feminine sexual behavior. Five hours following progesterone treatment, scopolamine HCl, a muscarinic receptor blocker, was infused intraventricularly. Infusion of scopolamine resulted in a transient, dose-related reduction in lordosis over the 3 concentrations used (5.0, 7.5, 10.0 µg/cannula in 0.5 µl volumes). Greatest reductions were observed 15 minutes after infusion.

These results lend further support to the concept that cholinergic brain mechanisms may mediate hormonal influences on sexual behavior in female rats.

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19.11 INTRACEREBRAL ANISOMYCIN BLOCKADE OF PROGESTERONE FACILITATED ESTROUS BEHAVIOR IN RATS IS SITE SPECIFIC. Jeffrey H. Glaser*, Susan C. Cislo*, and Ronald J. Barfield* (SPON: A. Hyndman). Department of Biological Sciences, Livingston Campus Rutgers University, New Brunswick, NJ 08903

The mechanism of progesterone (P) action in the facilitation of estrous behavior in estrogen-primed rats is not known. The existence, however, of estrogen-induced progestin receptors that translocate to the nucleus following P administration suggests that P may act via a genomic protein synthetic mechanism. Furthermore, whereas evidence for the neural site of P action in estrous behavior implicates the ventromedial hypothalamus (VMH), other sites including the preoptic area (POA) and the midbrain (MB) have also been implicated. Recently, Rainbow et al. (Br. Res. 233,1982) reported that VMH implants of the protein synthesis inhibitor anisomycin (ANI) inhibited the P facilitation of estrous behavior; these authors, however, did not investigate other brain sites, nor were nonspecific behavioral effects assessed. The present study was carried out in order to examine the effects of intracerebral implants of ANI in the VMH, POA, and MB on the facilitation of estrous behavior by progesterone.

Female Long-Evans rats (220-250g) were stereotaxically outfitted with 23 gauge bilateral guide cannulae directed towards the VMH (N=24), POA (N=8), and MB (interpeduncular nucleus, N=9). Following ovariectomy and insertion of sc 5% estradiol 17-beta silastic capsules for estrogen priming, animals received two tests in a counterbalanced design. Thirty minutes following the lowering of 28 gauge inner cannulae, which were either filled with ANI or blank, all animals were injected with 500 ug P sc. Cannulae were removed 2 h after P, and at 3 h several animals from each group were given a general activity test. At 4 h all animals were tested for estrous behavior with a vigorous male. Lordosis quotients (LQ) and solicitation scores (SS = # of darts and hops/ 10 mount test) were recorded.

Results showed that the anisomycin blockade of the progesterone activation of estrous behavior is site specific. Animals with ANI placed in the VMH showed little sexual behavior 4 h following P treatment (LQ=30.5±5.8; SS=1.8±.64; 29% proceptive). In contrast, animals with ANI placements in the POA (LQ=77.5±7.3; SS=15.6±3.0; 100% proceptive) and the MB (LQ=87.8±4.0; SS=10.2±2.1; 89% proceptive) were relatively unaffected. All animals appeared to be healthy, and no deficits in general activity were observed. Findings from any behavioral study using protein synthesis inhibitors must be viewed with caution. Nevertheless, the results of this study provide additional support for the essential role of the VMH as the primary site of P action, and they are consistent with the hypothesis that P acts to promote estrous behavior via a protein synthetic mechanism.

19.12 NORMAL DIFFERENTIATION OF SEXUAL BEHAVIOR IN MALE FERRETS DESPITE NEONATAL INHIBITION OF BRAIN AROMATASE OR 5α-REDUCTASE ACTIVITY. M. J. Baum, J. A. Canick*, M. S. Erskine*, C. A. Gallagher*, and J. G. Shim*. Dept. of Nutrition, M.I.T., Cambridge, MA 02139 and LHRRB, Harvard Medical School, Boston, MA 02115.

Male ferrets received s.c. implants of silastic capsules containing either the aromatase inhibitor, androst-1,4,6-triene-3, 17-dione (ATD), the 5α-reductase inhibitor, testosterone-17β-carboxylic acid (17βC), or no hormone for 15 days beginning at birth; an additional group of females received empty capsules during the same neonatal period. All animals were gonadectomized at 11 weeks and then were tested for masculine sexual behavior after a counterbalanced sequence of treatments with testosterone, estradiol, and 5α-dihydrotestosterone. Regardless of the neonatal treatment received, all groups of male ferrets displayed significantly higher levels of neck grip, mounting, and pelvic thrusting behavior than control females in adult tests. The three groups of males as well as the females all showed equivalent, dose-dependent increments in receptive behavior after administration of estradiol benzoate. Additional groups of newborn male and female ferrets were implanted s.c. with capsules containing ATD, 17βC, or no hormone, and were later killed on postnatal day 7. Administration of ATD, but not 17βC, strongly inhibited aromatase activity in hypothalamus + preoptic area (H + POA). The activity of 5α-reductase was very low in both H + POA and in cerebral cortex regardless of neonatal treatment. Neonatal administration of 17βC, but not ATD, caused a further reduction of 5α-reductase activity in cortex. Plasma concentrations of testosterone were equivalent on postnatal day 7 in males given ATD, 17βC, or no hormone at birth. The results suggest that behavioral defeminization fails to occur in ferrets of either sex whereas behavioral masculinization occurs in the male in response to the neonatal action of testosterone itself, as opposed to its estrogenic or 5α-reduced androgenic metabolites.

19.13 ANDROGEN AND ESTROGEN RECEPTORS IN DEVELOPING FERRET BRAIN. C. C. Vito, M. J. Baum, T. O. Fox, C. A. Gallagher* and M. S. Kindy*. Dept. of Neuropathol., Harvard Med. Sch., Boston, MA 02115 and Dept. of Nutrition, M.I.T., Cambridge, MA 02139.

Sexual differentiation of ferret brain appears to be developmentally and behaviorally distinct from that of rodents (mice, rats, hamsters) in that organization of male coital behavior is primarily modulated by testosterone rather than estrogen. In ferrets, the "critical period" of behavioral differentiation appears to be entirely neonatal, and sex hormones affect only masculinization, not defeminization, of adult coital behavior (Baum et al., Endocrinology, in press, 1982).

We investigated the possibility that the sex hormone receptor mechanisms in ferret brain are biochemically and/or developmentally distinct from that in rodents. Using DNA-cellulose affinity chromatography (Vito and Fox, Dev. Brain Res. 2: 97-110, 1982), we have characterized androgen and estrogen receptors in ferret brain 5 days before birth (42-day gestation period), at postnatal days 7 and 15, and in adulthood. Biochemically, both the androgen- and estrogen-binding activities in cytosol extracts of anterior hypothalamus-preoptic area, medial basal hypothalamus, temporal lobe and cortex at all ages tested are similar to those in rodent brain: Androgen-binding activities exhibit elution maxima at 120 - 140 mM NaCl while estrogen-binding activities exhibit elution maxima at 200 - 220 mM NaCl, and putative adult androgen receptors bind both dihydrotestosterone and testosterone while estrogen receptors bind both estradiol and diethylstilbestrol.

In contrast, the developmental profiles of androgen and estrogen receptors in ferret brain appear to differ from those in rodent brain. In ferret hypothalamus and preoptic area (H + POA), androgen receptor concentrations are relatively high prenatally and remain high through the "critical period" and into adulthood, whereas in mice and rats androgen receptor concentrations are lower at prenatal ages and increase gradually to similar levels in adulthood. Likewise, estrogen receptor concentrations in ferret H + POA are high prenatally and remain high through early postnatal ages (see also Holbrook and Baum, in press, 1982); these levels are higher than those detected at any age in either mouse or rat H + POA. In ferrets, estrogen receptor concentrations are higher than those of androgen receptor at prenatal and early postnatal ages while both approach more similar levels in adulthood. To date, we have not detected sex differences in either receptor system.

The persistently high concentration of androgen receptors, together with the changing ratio of estrogen to androgen receptor, in postnatal H + POA may contribute to the ferrets' high responsiveness to testosterone and to the timing of its neonatal "critical period."

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19.14 COMPETITION FOR ANDROGEN RECEPTOR SITES BY FLUTAMIDE AND ITS METABOLITES. B. Gladue* (SPON: R.E. Whalen). Long Island Research Institute, SUNY at Stony Brook, NY 11794.

Flutamide (FLU; 4'-nitro-3'-trifluoromethylisobutyranilide), the non-steroidal antiandrogen has been shown to interfere with testosterone-induced behavioral sex differentiation (Clemens, et al. Horm. Behav. 10,40,1978) and adult sexual behavior (Gladue, B.A. and Clemens, L.G. Endo. 106,1917,1980). Yet, in vitro studies demonstrate poor inhibitory properties of FLU upon androgen binding to steroid receptors. Possibly FLU action upon CNS tissues may require in vivo conversion to a more active metabolite with greater inhibitory properties. To test this hypothesis, metabolites of FLU were evaluated for their relative effectiveness at competing with ^{3}H-DHT for androgen receptor sites in hypothalamic-preoptic area (HYPOA) and cerebral cortex (CTX) tissues of adult short-term castrated male rats.

Cytosol extracts were incubated (4° C) for 18 hrs with 2 nM ^{3}H-DHT alone or with 200 nM DHT, FLU, the hydroxy metabolites of flutamide, SCH 16423 (α,α,α-trifluoro-2-methyl-4'-nitro-m-lactotoluidide) and RU 23908 (5,5-dimethyl-3-[4-nitro-3-(trifluoromethyl)phenyl-2,4-imidazolidine-dione). For comparison, a steroidal antiandrogen R 2956 (17β-hydroxy-2,2,17-trimethyloestra-4,9,11-triene-3-one) was also evaluated for its ability to compete for ^{3}H-DHT. Bound ^{3}H-DHT was measured after separation with Sephadex LH-20 chromatography. Percentage inhibition was computed on the basis of relative effectiveness to that of 200nM DHT, which was arbitrarily designated as 100%.

COMPETING SUBSTANCE	HYPOA	CTX
DHT	100	100
FLU	2	8
SCH 16423	40	50
RU 23908	24	36
R 2956	55	55

These data demonstrate that the metabolites of Flutamide are more effective at competing for androgen receptor sites than that of Flutamide itself, in both HYPOA and CTX. Therefore, the antiandrogenic actions of FLU may require in vivo metabolism to a more active form suggesting that FLU interference with testosterone-mediated processes may result from one of its metabolites.

20.1 OPIOID PEPTIDES MAY MEDIATE THE IMMUNOSUPPRESSIVE EFFECT OF STRESS. Y. Shavit, J.W. Lewis, G.W. Terman*, R.P. Gale*, and J.C. Liebeskind. Departments of Psychology and Medicine, UCLA, Los Angeles, CA 90024.

A number of studies indicate that stress can modulate the responsiveness of the immune system (see review by Solomon and Amkraut, 1981). Several findings suggested to us that this modulatory effect may be mediated by opioid peptides released during stress. Inescapable, but not escapable, stress causes opioid mediated analgesia in rats (Maier et al., 1980). Inescapable, but not escapable, stress can also enhance tumor development in animals (Sklar and Anisman, 1979; Visintainer et al., 1982). In addition, opiate antagonists were found to inhibit tumor growth and prolong survival of animals given tumors (Aylsworth et al., 1979; Zagon et al., 1981). Taken together, these results suggest the involvement of opioid peptides in the development of tumors, possibly via modulation of the immune system. To test this hypothesis we employed two types of footshock stress, one shown to induce an opioid, the other a nonopioid, form of analgesia (Lewis et al., 1980; 1981). We examined the effects of these stressors on the reactivity of the immune system to two different lymphocyte mitogens.

Rats were subjected to either the opioid (2.5 mA, 1 sec every 5 sec, for 20 min) or the nonopioid (2.5 mA, for 3 min continuously) footshock stress paradigms. Two or 24 hrs after stress, rats were exsanguinated by heart puncture. The whole blood was separated by Ficoll-hypague gradient centrifugation and the mononuclear cell fraction was recovered. 2×10^5 cells were cocultivated with the lymphocyte mitogens Con A (1 µg) or PHA (1:20), and cell proliferation (DNA synthesis) was determined 72 hrs later by the incorporation of ^{3}H-thymidine.

The opioid stress paradigm significantly decreased the response to both mitogens (to ca. 35% of control values) in cells obtained 2 hrs after stress. The response of cells obtained 24 hrs later was slightly elevated compared to controls (143% for con A, 134% for PHA). In contrast, the response of cells obtained 2 hrs after the nonopioid stress paradigm did not differ from the control response. Finally, preliminary data indicate that the immunosuppressive effect of the opioid type of stress is blocked by the opiate antagonist naltrexone (10 mg/kg). These results provide evidence that the immunosuppressive effect of stress is mediated, at least in part, by opioid peptides. (Supported by a gift from the Brotman Foundation.)

20.2 AUTONOMIC INNERVATION OF THYMIC/LYMPHOID TISSUE IN THE THORACIC CAVITY OF NUDE MICE. A. Lapa*, M. Teiche*, K. Bulloch. Dept. of Neurology, SUNY-Stony Brook. Sch. of Med., Stony Brook, NY 11794

During the perinatal period, the development of integrity in the function of the thymus gland is crucial to the formation of a competent immune system and the neuroendocrine axis. Recent studies now indicate that differentiation of the thymus occurs after it is innervated by fiber of the vagus nerve suggesting a role for innervation in the development of this gland. To test this hypothesis, we have examined innervation of rudimentary structures of putative thymic origin within the thoracic cavity of nude mice. This mutant was chosen because alterations in thymic development occur at a time concomitant with the innervation of the gland by vagal fibers. Acetylcholinesterase (AchE) histochemistry was used to demonstrate parasympathetic terminals in the nude thymic rudiment and sympathetic innervation was shown by glyoxylic acid fluorescence histochemistry techniques. Routine histology was used to examine the relationship of peripheral fibers and ganglia to lymphoid tissue.

Nude mice have a polycystic organ and numerous aberrant lymphoid accumulations (LA) and lymph nodes (LN) within the thoracic cavity. The lymphoid tissue is always found in close association with ganglia, nerve fibers, or both. This association also is found in wild type mice. In many cases,the morphology of the LA resembles that of the LN,however, they fail to demonstrate lymphatic afferent drainage. The sympathetic (CA) and parasympathetic (AchE) innervation of nude LN is similar to the wildtype mouse. CA fibers are evident within the capsule, along the vasculture and in discrete areas within the interior of the nodes. Cholinergic fibers are also present in the capsule along the vasculature and "in transit" along the outer parenchama of the node. Very few CA and cholinergic fibers are found within the stroma of LAs in either nude or wildtype mouse. The nude polycystic organ also demonstrates very limited innervation by CA or AchE histochemical techniques. No fibers are evident among the cells of the cyst nor are any present in its outer boarders. The lack of cholinergic innervation in the polycystic organ and those LA reported to be thymic rudiments (Holub, 1975) is in accord with the view that vagal innervation plays a role in thymic development. In addition, the observation that many LN and LA tissue develop in association with ANS fibers and ganglia suggest the organization of other immune system tissue also may be dependent on the nervous system.

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20.3 BINDING CHARACTERISTICS OF A NOVEL CHOLINERGIC RECEPTOR SITE ON MURINE LYMPHOCYTES. John J. Grayhack*, Samir F. Atweh, and David P. Richman*(SPON: M.A. Hollyday) Dept. of Neurology, The University of Chicago, Chicago, IL 60637.

Following observations of cholinergic receptors on lymphocytes we have shown contrasting functional responses of these cells to carbamylcholine (Richman, D.P. and Arnason, B.G.W., Proc. Natl. Acad. Sci.USA 76, 4632). The stimulatory response is blocked by muscarinic antagonists and the suppressive response by nicotinic antagonists. In light of these findings, we have investigated a cholinergic binding site on isolated adult mouse spleen lymphocytes, demonstrated with the use of the muscarinic antagonist ^{3}H-quinuclidinly benzilate (^{3}H-QNB). This binding site has shown affinities and specificity differing from muscarinic receptor sites in rat brain tissue. In our mouse spleen lymphocytes, binding was displaceable by both muscarinic and nicotinic antagonists. In view of this unusual specificity we were led to investigate the kinetics and displacement of this binding site.

Lymphocytes were prepared from the spleens of Swiss mice, and the red cells were separated either by ammonium chloride lysis at 4°C or by centrifugation on a Ficoll-hypaque discontinuous gradient. Adherent cells were removed by incubation on plastic. The lymphocyte fraction was incubated with 2nM ^{3}H-QNB in phosphate buffered saline for 30 minutes at room temperature and free ligand removed by either filtration or centrifugation. 80-90% of the ^{3}H-QNB binding was displaceable by cholinergic ligands. The possibility of QNB uptake was discounted by the demonstration of binding to membrane preparations and by the rapid rate of dissociation of the bound ligand. Multiple cholinergic ligands competed for binding with ^{3}H-QNB including atropine, scopolamine, acetylcholine, α-bungarotoxin, and d-tubocurarine. Unlabeled QNB competed for binding in a biphasic manner suggesting two binding sites with IC_{50}'s of 0.1µM and 5µM respectively. Atropine and d-tubocurarine displaced ^{3}H-QNB with IC_{50}'s of 50µM and 70µM respectively. Under similar conditions, binding of ^{3}H-QNB to rat brain membranes was displaced with unlabeled QNB and atropine with IC_{50}'s of 1 and 7nM while d-tubocurarine had an IC_{50} of 10µM.

In summary, we have demonstrated a QNB binding site on splenic murine lymphocytes which is competed for by several cholinergic ligands. It differs from classical muscarinic sites by its relatively low affinity to QNB and by the fact that muscarinic and nicotinic antagonists are almost equipotent in displacing ^{3}H-QNB. The lymphocytic receptor is of unknown physiological significance but any such receptor may be functionally relevant and provide a means of selective pharmacological control of immunologic response or pathology. This consideration is especially true in the case of myasthenia gravis, in which an autoimmune response against receptors is associated with reduced suppressor cell function.

20.4 INCREASED β-ADRENERGIC RECEPTORS ON SPLEEN LYMPHOCYTES FROM MICE LACKING PERIPHERAL SYMPATHETIC NERVOUS SYSTEM. K. Miles*, G. Otten*, S. Atweh*, E. Chelmicka-Schorr* and B. Arnason. Dept. of Neurology, Univ. of Chicago, Chicago, IL 60637.

A role for the sympathetic nervous system in regulating the murine immune response in vivo has been demonstrated previously. We reported a significantly enhanced antibody forming cell response to 2 thymus-independent antigens in mice treated with 6-hydroxydopamine to destroy peripheral sympathetic nerve endings (axotomy). (Miles, K. et al, J. Neuroimmunol. 1:101, 1981).

Recently we studied lymphocyte β-adrenergic receptors to probe the cellular events responsible for this increased antibody production in axotomized mice. β-adrenergic receptors on axotomized and control A/J male mouse spleen lymphocytes were quantitated using ^{3}H-dihydroalprenolol (DHA). Whole cells, nonadherent to plastic culture dishes to exclude macrophages and erythrocyte depleted either by NH_4Cl lysis at 4°C or by centrifugation on a Ficoll-hypaque gradient, were incubated with ^{3}H-DHA in phosphate buffered saline for 30 minutes at 25°C. The cells and bound ligand were then separated either by rapid filtration or by centrifugation. Specific binding, displaceable by 10^{-4}M propranolol, accounted for 80% of the total binding. Spleen lymphocytes from axotomized animals show a 40-60% ($p < .01$) increase in ^{3}H-DHA binding. Scatchard analysis reveals an increase in the number of binding sites.

To examine the correlation between receptor density and lymphocyte function, we separated control mouse spleen lymphocytes into B and T subpopulations on nylon wool columns and then measured β-adrenergic receptors. A 2-3 fold enrichment of specific binding was detected on the B compared to T cell populations. We attempted to distinguish whether axotomized mouse lymphocytes have more receptors per cell or whether a population shift occurred in the direction of more B cells which could also explain the increased number of binding sites. We labeled the B cells of spleen lymphocytes with fluoresceinated goat anti-mouse Ig. Using flow cytometry to detect and quantitate small positive cells, we report a 25% decrease in the number of B cells present in axotomized mouse spleen lymphocyte populations.

The reduction of the higher density receptor B cell population in axotomized mice further enhances the significance of the difference in receptor number of axotomized compared to control mouse lymphocytes. Recent data suggests that both T and B cells in axotomized mice have increased receptor sites. We postulate a denervation hypersensitivity event occurring after axotomy. These findings illustrate a further link between sympathetic nervous system activity and immune function.

20.5 A LIGHT AND ULTRASTRUCTURAL ANALYSIS OF INNERVATION OF THE THYMUS GLAND DURING THE PERINATAL PERIOD. K. Bulloch. Dept. of Neurology School of Medicine. SUNY at Stony Brook, Stony Brook, NY 11794.

Recent studies reveal the thymus receives direct projections from the CNS prior to birth. (Bulloch & Moore, 1981, Bulloch & Loy, 1981). In order to further characterize this innervation, a qualitative light and ultrastructural analysis of the thymus of the C57BL/6J mouse during embryonic and perinatal development was undertaken. Routine light and ultrastructural histology was used to examine the development of the thymus from E-13 - P-3.

Light and ultrastructural analysis revealed that the thymus is innervated by the Vagus prior to E-13. The nerve penetrates the thymus in the cervical area and descends with the gland into the thoracic cavity. Fibers are distributed throughout, terminating in areas that define the future cortico-medullary boundaries and to a discrete layer of cells adjacent to the capsule. Growth cones, most likely derived from the Vagal fibers, are common within the thymus through E-18, and become less frequent as the mouse approaches P-3. Some of these cones form synapses with each other and contain clear spherical vesicles 40-50 nm at presynaptic terminals. Other fibers are found to form synapses with processes and soma of thymic stroma cells. Two cell types are associated in areas of dense synaptic contacts. A large, clear cytoplasmic cell at the developing cortico-medullary boundary and a dense "reticular-like" cell located at the outer perimeter of the developing cortex. Vescicular content of the fibers and boutons associated with these cells vary. Large dense core vesicles 100 nm, lucent pleomorphic 50-60 nm by 25 nm vesicles as well as the clear spherical vesicle are found in synaptic profiles. Large dense core vesicles predominantly are found in boutons containing small clear spherical vesicles. All three types of vesicles are evident in various combinations in either clear or osmophilic axons and terminals. No pattern of synaptogenesis on any given cell type is apparent though synaptic contacts are more common on cell processes and other axons than on cell soma.

The temporal and terminal distribution of vagal fibers within the thymus precedes its morphological and functional differentiation. These data suggest a role for the nervous system in thymic development.

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21.1 FUNCTIONAL MAPPING OF VAGAL TRUNK NEURONS IN THE DECENTRALIZED STELLATE AND MIDDLE CERVICAL GANGLIA OF THE DOG USING [^{14}C] 2-DEOXYGLUCOSE. D.R. Kostreva, J.A. Armour* and Z.J. Bosnjak. Depts. Anesthesiol. and Physiol., Med. Col. Wis. and Wood VA Med. Ctr., Milw., Wis. 53193 and Dept. Physiol., Dalhousie U., Halifax, Nova Scotia B3H 4H7.

Recent extracellular and intracellular electrophysiological and HRP studies of the stellate (SG) and middle cervical ganglia (MCG) of the cat and dog indicate that some cardiopulmonary reflexes may be mediated by these thoracic ganglia. (Bosnjak, Am. J. Physiol. 242:R237-R243, 1982; J. Physiol. 324:273-283, 1982), (Armour, J. Comp. Neurol. 202:169-184, 1981; Cardiol. 61:309-328, 1976). The functional neuroanatomical mapping technique of Sokoloff using [^{14}C] deoxyglucose (DG) was used to determine if focal changes in DG uptake could be elicited in the decentralized SG and MCG during electrical stimulation of the cut central end of the left vagus at the level of the heart. Adult dogs 3-4 kg were anesthetized with sodium pentobarbital (35mg/kg i.v.), intubated and placed on positive pressure ventilation. Systemic blood pressure and electrocardiogram were recorded using a polygraph. The left SG and MCG were decentralized by sectioning all of the vagal and sympathetic neural connections with these ganglia except for the ansae subclaviae and cardiopulmonary nerves caudal to these ganglia. The left vagal trunk sectioned just above the heart, was partially desheathed and the central end was stimulated electrically using bipolar electrodes at a frequency of 10 Hz, 0.5 ms pulse width, and a current strength of 5 mA. Prior to the initiation of stimulation, a single bolus injection of DG (Pathfinders Labs; 110 uCi/kg) was administered i.v. Periodic stimulation of the vagal trunk, one to two minutes on and one minute off, was continued throughout the 45 minute experimental period. The SG and MCG were then removed together and frozen sections were made at 20 micron increments. The sections were then covered with film for autoradiography. After a 12 day exposure, the films were developed and selected corresponding sections were Nissl stained. The results demonstrate that electrical stimulation of the cut central end of the left vagal trunk at the level of the heart can produce focal increases in DG uptake within the SG, but not within the MCG. However, in one animal, a cluster of ganglion cells located outside of the MCG had markedly increased DG uptake as compared to the MCG. It is not possible in this study to determine whether the increases in DG uptake of the SG are due to the orthograde activation of afferent or to retrograde activation of efferent neurons. However, this data does strongly suggest a functional connectivity of the SG with the vagal trunk caudal to the MCG, without a functional connectivity with the MCG. (Supported by the Dept. Anesthesiol. Medical College of Wisconsin and the Wood VA Medical Center).

21.2 DIFFERENCES IN SPONTANEOUS AND REFLEX ACTIVITY AMONG RENAL, CARDIAC, AND SPLENIC SYMPATHETIC NERVES FOLLOWING HIGH CERVICAL SPINAL CORD TRANSECTION. R.L. Meckler, H.K. Fry*, and L.C. Weaver. Dept. of Physiol., Mich. State Univ., E. Lansing MI 48824

Spontaneous activity of sympathetic nerves is depressed initially following high cervical spinal cord transection but gradually increases with time. Uniformity of the effects of spinal cord transection on basal discharge of different sympathetic nerves has not been investigated. In addition, although increasing systemic blood pressure is known to cause changes in sympathetic discharge in spinal animals, the homogeneity of such responses in different sympathetic nerves has not been assessed. Therefore, uniformity of sympathetic responses was evaluated in alpha-chloralose anesthetized cats in which the IXth and Xth cranial nerves had been severed. Sympathetic activity was recorded simultaneously from pairs of nerves (renal and cardiac, renal and splenic, cardiac and splenic) during and after spinal transection at the 1st cervical segment. Blood pressure was maintained at not less than 70 mm Hg by intravenous infusion of phenylephrine when necessary.

Immediately following spinal transection, renal nerves were completely quiescent in 11 of 13 cats evaluated. In the remaining 2 cats renal nerve activity was depressed to less than 50% of control discharge. In contrast, splenic nerves ceased firing after transection in only 1 of 7 cats, activity was depressed to less than 50% in 3 cats and was unchanged or increased in 3 cats. Similarly, cardiac nerve activity was abolished immediately in only 1 of 9 cats, was depressed to less than 50% in 4 cats and was unaffected or increased in 4 cats. Recovery of spontaneous discharge also differed among the 3 populations of nerves.

Increasing systemic arterial pressure from 78±3 to 124±8 mm Hg by injection of pressor agents in 6 Cl spinalized cats produced a 57% decrease in cardiac sympathetic activity but only a 25% decrease in renal nerve discharge. The differences in these responses could not be attributed to unequal rates of spontaneous discharge in the 2 nerves. The sympatho-inhibition may have originated in part from activation of cardiac spinal afferent neurons. In 3 spinal cats stimulation of epicardial receptors by bradykinin caused 39% inhibition of cardiac nerve activity whereas renal nerve activity was excited or unaffected by this stimulus.

These results indicate that cardiac, splenic and renal sympathetic nerves are not dependent to the same extent upon supraspinal drive for basal excitability. Moreover, in spinal cats, cardiac and renal nerves were unequally sensitive to inhibitory influences initiated by increasing systemic arterial pressure.

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21.3 VENOUS AFFERENT CONTRIBUTION TO DISTRIBUTED NEURAL CONTROL OF THE MUSCLE-TONUS VENOPRESSOR MECHANISM. F.J. Thompson and B.J. Yates*. Dept. of Neuroscience, Univ. of Fla. Coll. of Med., Gainesville, FL 32610.

A problem of major significance in the neural control of the cardiovascular system is how the central nervous system controls venous return during and subsequent to changes in posture. The CNS mediated change in skin and visceral venous capacitance in response to central baroreceptor inputs has emphasized a dynamic role for these two venous components in the regulation of circulation. Understanding of the role of peripheral venous afferents in the neural control of circulation has been prevented by the lack of details regarding their properties and connections in the CNS.

Although it is known that orthostasis leads to blood pooling in the long limb veins and subsequently, to volume pooling in skeletal muscle veins, reflex control of the capacitance of the skeletal muscle venous reservoir has not been described in the classical literature.

Yandel Henderson and colleagues revealed that venous pressure in general and intramuscular venous capacitance in particular was supported by muscle tonus, and described this functional relationship as the venopressor mechanism. They recognized that the central nervous system controlled muscle tonus, but were unable to identify an input variable which was specifically related to this mechanism.

Recently, details of a peripheral venous afferent input to the spinal cord (Thompson and Barnes, 1979) to the cerebral cortex (Thompson, Lerner, Fields and Blackwelder, 1980) and to somatic motoneurons (Thompson, Barnes and Wald, 1982) were reported. The connections revealed in these studies provide the neural substrate for venous afferent elicited influences on skeletal muscle tone.

In experiments carried out on decerebrate cats spinal at T_{10}, we have observed that electrical stimulation of femoral venous afferents produced a marked increase in hindlimb skeletal muscle tone measured by recording both intramuscular pressure and muscle contraction tension.

In addition we have observed that distention of segments of limb veins in decerebrate spinal cats produced facilitation of lumbar motor neurons. Limb vein distention was also shown to elicit activity of neurons in the cerebral cortex with the principal focus in the limb motor cortex in cats anesthetized with α-chloralose.

The authors propose that the venous afferents form a major contribution to the central integration and control of intramuscular venous capacitance. The ogranization of venous afferent CNS connections as a distributed system will be discussed.

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21.4 DIFFERENTIAL SYMPATHETIC REFLEXES INITIATED BY DISTENSION OF THE URINARY BLADDER AND DUODENUM. H.K. Fry* and L.C. Weaver (SPON: J.R. Hoffert). Dept. of Physiol., Mich. State Univ., E. Lansing MI

Distension of the urinary bladder and duodenum stimulates visceral afferent neurons to elicit sympathetic reflexes. This study was undertaken to determine the uniformity of responses in splanchnic sympathetic outflow to these visceral afferent stimuli. Renal and splenic sympathetic reflexes were investigated in alpha chloralose anesthetized cats in which carotid sinus and aortic depressor nerves had been severed. Activity of these multifiber sympathetic efferent nerves was recorded simultaneously. Balloon-tipped catheters were inserted into the urinary bladder and duodenum through small incisions. Reflex responses to distension of these organs were compared prior to and following vagotomy. Distension of the urinary bladder caused pressor responses and excitation of renal nerve activity whereas, splenic nerve activity was either excited or inhibited by this stimulus. Excitation of renal nerve discharge was greater than or equal to the increase observed in splenic nerve activity. The excitatory response to bladder distension was observed in both sympathetic nerves following vagotomy suggesting that this reflex was initiated at least in part by spinal afferent neurons. Distension of the duodenum initiated pressor responses and excitatory or inhibitory reflexes in renal and splenic nerves. Opposite responses were sometimes but not always observed in the two nerves. Excitation of renal nerve activity tended to be greater than that of splenic nerve activity and reciprocally, splenic nerve activity tended to be more inhibited by duodenal distension than was renal sympathetic discharge.

In summary, distention of the urinary bladder and duodenum tended to cause greater excitation of renal than splenic sympathetic discharge. These findings can be contrasted with results obtained in previous studies completed in our laboratory in which stimulation of other visceral afferent neurons caused greater excitation of splenic than renal nerve activity (Meckler et al., Neurosci 7:366, 1981). Thus, these results provide further evidence that the sympathetic nervous system is capable of non-uniform discharge and that variations in specific reflex patterns depend upon the source of afferent initiation. Support: NIH grant HL21436.

21.5 CENTRAL ORGANIZATION OF CAROTID BARORECEPTOR AND CHEMORECEPTOR PROJECTIONS IN THE CAT. S. Donoghue*, R.B. Felder*, D. Jordan* and K.M. Spyer* (SPON: M. Stewart). Department of Physiology, Royal Free Hospital School of Medicine, London, England and the Cardiovascular Center, University of Iowa, Iowa City, Iowa.

Afferent fibers in the carotid sinus nerve are known to terminate centrally in the nucleus of the tractus solitarius (NTS). However, the organization within the NTS of axons and terminal beds of specific types of afferent fibers from the carotid sinus nerve has not previously been studied.

In anesthetized artificially ventilated cats, we obtained extracellular recordings from single baroreceptor or chemoreceptor cell bodies in the petrosal ganglion. Baroreceptor units were identified by pulse rhythmical activity which was abolished by ipsilateral common carotid occlusion; chemoreceptor units were identified by increased activity in response to 30 seconds of ipsilateral common carotid occlusion or to increasing end-tidal CO_2. The brain stem projections of cells having a stable signal:noise ratio were systematically investigated using antidromic stimulation techniques previously established in this laboratory (Donoghue, S., Garcia, M., Jordan, D. and Spyer, K.M., J Physiol 322:337-352, 1982).

Baroreceptors with myelinated axons (n=6) and nonmyelinated axons (n=3) projected to medial and lateral subnuclei of NTS between 0-3 mm rostral to obex. Chemoreceptors with unmyelinated axons (n=6) terminated mainly in medial subnuclei of NTS 1.5-3.5 mm rostral to obex. Myelinated chemoreceptor units were not observed. Chemoreceptors were characterized by discrete terminations close to the main axon, while baroreceptors had widespread areas of branching and termination.

The differences in pattern of projection of carotid sinus baroreceptors and chemoreceptors suggest that these two groups of sensory receptors influence different cell populations within the NTS.

21.6 NEURONS IN THE DOG'S AREA POSTREMA ARE ESSENTIAL FOR ITS SYMPATHOFACILITATIVE CARDIOVASCULAR ACTIONS. Karen L. Barnes and Carlos M. Ferrario. Cleveland Clinic Research Division, Cleveland, OH 44106.

We have previously shown that electrical stimulation of the dog's area postrema mimics the pressor response produced by intravertebral infusion of low-dose angiotensin II, causing an increase in mean arterial pressure associated with onset tachycardia and increased peripheral resistance (Barnes, K.L., Ferrario, C.M. and Conomy, J.P.: Circ. Res. 45: 136, 1979). In order to show that this pressor mechanism is initiated by neurons localized within the area postrema it was necessary to eliminate the possibility that the pressor pathway within the area postrema consisted of efferent fibers of passage from some distant pressor region. Thus we examined the characteristics of the area postrema pressor response before and after: 1) surgical transection of the brainstem at the midcollicular level, and 2) microinjection of kainic acid into the area postrema. In morphine-chloralose anesthetized dogs the area postrema was stimulated with 20 sec trains of 0.2 msec pulses at 50 Hz and 20, 50 and 80 μA (threshold 10 μA). Midcollicular transection in 3 dogs did not alter resting mean arterial pressure or heart rate (before: 118 ± 9 mmHg, 55 ± 6 beats/min; after: 110 ± 7 mmHg, 60 ± 9 beats/min) or the magnitude of the area postrema pressor responses at 20, 50 and 80 μA. Peak increases in mean pressure were 19 ± 1, 30 ± 3 and 40 ± 4 mmHg in the intact state and 20 ± 1, 32 ± 3 and 46 ± 6 mmHg after transection; peak heart rate increases were 15 ± 4, 31 ± 7 and 50 ± 10 beats/min before and 18 ± 6, 26 ± 5 and 42 ± 5 beats/min after transection. In 4 other dogs kainic acid (10 nmoles in 1 μl phosphate buffered saline) was microinjected into the right area postrema, and 1 μl vehicle into the left area postrema. Mean arterial pressure rose to 227 ± 7 mmHg, and heart rate to 97 ± 3 beats/min, but returned to control values during the next two hours. When electrical stimulation was repeated, the pressor responses were unaltered in the left area postrema (vehicle) and abolished in the right area postrema after kainic acid. The site and extent of damage produced by the kainic acid were histologically verified. These results provide evidence that the area postrema pressor pathway is initiated by neurons within the area postrema, rather than by fibers of passage from other pressor centers. Supported by grants from NHLBI (HL-6835) and the Reinberger Foundation.

21.7 CARDIOVASCULAR RESPONSES ELICITED BY ELECTRICAL STIMULATION OF MIDLINE NEOCORTEX AND THE MEDIODORSAL AND ANTERIOR THALAMIC NUCLEI OF THE CONSCIOUS RABBIT. Shirley L. Buchanan, and D. A. Powell, Neuroscience Lab., VA Hospital and Univ. of South Carolina, and James Buggy, Dept. of Physiology, Univ. of South Carolina School of Medicine, Columbia, S.C.

Previous studies revealed that electrical stimulation of midline neocortex (precentralis agranularis) in conscious rabbits elicited pronounced bradycardia and vasodepressor responses. The bradycardia was abolished by atropine but was unaffected by propranolol or phentolamine. In the present study, stimulation subsequent to the injection of 1.6 mCi/kg of ^{3}H-2-deoxyglucose resulted in increased metabolic activity, ipsilateral to the electrodes, in both anterior and mediodorsal nuclei of the thalamus. Other experiments mapped the dorsal thalamus, especially the MD and anterior nuclei, for cardiovascular responses elicited by electrical stimulation. Responses were assessed as a function of train duration, pulse frequency, and stimulus intensity. Respiration, EMG and gross motor activity were also recorded.

Electrical stimulation of the MD and anterior nuclei of the thalamus resulted in pronounced bradycardia, in some cases preceded by a brief heart rate acceleration. The accompanying BP response was in some cases a pressor response. Administration of atropine and/or propranolol suggested that although this response is primarily vagally mediated it may be at least partially determined by sympathetic inhibition. It was also shown that the bradycardiac response was not due to elicitation of the baroreceptor reflex, since α-receptor blockade (phentolamine) abolished the BP response but left the bradycardiac response intact. Stimulation in other regions of the thalamus (usually more anterior) resulted in monotonic BP depressor responses and HR decelerations. Again, the HR decelerations were atropine dependent, but were unaffected by propranolol or phentolamine. These cardiovascular responses were in all cases accompanied by increases in respiration frequency and decreases in depth. At stimulus parameters which elicited pronounced cardiac and vascular changes, no movement occurred.

These studies suggest that the bradycardia and depressor responses elicited by anterior midline cortical stimulation are related to pathways from the anterior cingulate to the anterior and MD nuclei of the thalamus. Other types of cardiovascular responses are generated in MD nuclei, but the nature and behavioral significance of these responses is unclear at the present time.

21.8 CENTRAL CONTROL OF THE CARDIOVASCULAR AND RESPIRATORY COMPONENTS OF THE CEREBRAL ISCHEMIC RESPONSE (CIR). James R. Haselton*, Howard H. Ellenberger*, Philip M. McCabe, and Neil Schneiderman. Program in Behavioral Medicine, Dept. Psych., Univ. of Miami, Coral Gables, FL 33124.

The CIR consists of elevated arterial blood pressure, profound bradycardia, apnea, and isoelectric cortical EEG. We have reliably reproduced this pattern in rabbits using bilateral occlusion of the vertebral and common carotid arteries. Each vertebral artery was occluded by packing cotton dental pellets into the transverse foramen of the C2 vertebra. The common carotid arteries were temporarily occluded to induce brief episodes of cerebral ischemia (CI).

Cervical vagotomy transformed the bradycardia component into tachycardia, indicating that the bradycardia is vagally mediated. Aortic nerve section attenuated, but did not abolish, the bradycardia component, suggesting that at least part of the cardiodeceleration is a centrally mediated primary bradycardia. Neither of these nerve sections altered the blood pressure or respiratory components of the CIR. Artificial ventilation and neuromuscular blockade failed to alter the heart rate and blood pressure components, demonstrating that these components are not secondary to the apnea. Previous work has shown that the CIR persists after pontomedullary transection, whereas spinal cord section at C1 abolishes the reflex hypertension. These observations indicate that the CIR is organized in the medulla. Extracellular recordings were made of medullary neurons before, during, and after repeated brief episodes of CI. Inspiratory neurons were found in the dorsal medulla that responded to bilateral vertebral and carotid occlusion with a decrease or cessation of firing. Conversely, cardiovascular-related neurons were found in the dorsal vagal nucleus that increased their discharge rate during the four-vessel occlusion.

CI episodes are a common risk following cardiac arrest or during profound hypotension. The four-vessel occlusion technique appears to be clinically interesting insofar as it permits venous drainage of the head to remain uncompromised. Present experiments directed towards understanding the neuronal control of the circulation in response to CI and its sequelae should be useful in elucidating the role of circulatory adaptations in reestablishing cerebral perfusion following CI.

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21.9 ALPHA ADRENERGIC RECEPTOR SITE BINDING IN THE FOREBRAIN AND BRAINSTEM OF THE DAHL RAT MODEL OF HYPERTENSION. E. Edwards*, J. McCaughran, Jr., R. Friedman*, J. Iwai* and N. Schechter. (Spon. A. Orr). Long Island Research Institute and Department of Psychiatry, SUNY at Stony Brook, NY 11794

The Dahl model of hypertension consists of two lines of rats: a salt sensitive or DS line which remains normotensive unless exposed to a high salt diet and a salt resistant or DR line which shows no change in blood pressure irrespective of salt intake. We determined the number and affinity of the α-adrenergic (α_1 and α_2) receptor sites in cortex, medulla and hypothalamus in DS and DR rats on both high and low salt diets. In addition, drug inhibition studies were performed to pharmacologically characterize the receptors.

After three weeks on their respective diets and following blood pressure determination, brain regions from DR and DS rats were homogenized and aliquots were incubated in the presence of ^{3}H-clonidine for α_2 receptors and ^{3}H-WB4101 for α_1 receptors. Non-specific binding was determined by parallel incubations with 10 μM and 100 μM (-) -norepinephrine respectively. A detailed method has been previously described (Mol. Pharmacol., 16, 1979, 57-60).

The high salt diet resulted in significant elevation in blood pressure in DS rats but not in DR rats. In the low salt condition where both lines are normotensive the DS rats had 40% fewer α_1 binding sites than DR rats in the hypothalamus. The DS rats fed high salt had even fewer α_1 binding sites than the low salt DS rats in the hypothalamus. There were no differences in the binding of the α_1 adrenergic ligand in any other brain regions. ^{3}H-clonidine binding to α_2 receptors showed no differences in any area examined in the four line x diet groups.

The affinity of both α_1 and α_2 receptors for ^{3}H-clonidine and ^{3}H-WB4101 was unaffected in all four groups. K_d values obtained from Scatchard plots were 2.84 ± .22 nM for clonidine and 0.46 ± .02 nM for WB4101. Pharmacological characterization of the α_1 adrenergic receptor revealed that the displaceability of ^{3}H-WB4101 is consistent with the specificity of the non-labeled ligand to react with α_1-receptors. IC_{50} values obtained from displacement curves followed the order: WB4101 > oxymetazoline > yohimbine > clonidine > propanolol. We have previously reported (Neuroscience meeting, 1981) an enhanced activity of the cholinergic system in the DS rats. The noradrenergic data presented here lead us to speculate that interactions between cholinergic and noradrenergic systems may play a role in the blood pressure regulation of DS and DR rats.

22.1 SPECIFICITY OF HYPOTHALAMIC-AUTONOMIC CONNECTIONS. C. B. Saper, Dept. of Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110

Hypothalamic neurons project directly to preganglionic cell groups in the medulla and spinal cord, but the functional specificity of these connections is not unknown. Electrophysiological studies indicate that certain functionally specific preganglionic pools (e.g., pupillodilator, vasoconstrictor, vesicoconstrictor) may be innervated by particular subsets of hypothalamic neurons. The specificity of hypothalamic-autonomic connections has therefore been investigated by the use of retrograde fluorescence double-label methods in the rat.

Microinjections of fast blue and nuclear yellow were placed through the dorsal column into the intermediate gray matter at different levels of the thoracic spinal cord in the rat. Only injections limited to the spinal gray matter of a single segment were considered. Absence of double-labeled neurons in the red nucleus, indicating lack of uptake of dye by lateral column fibers of passage, was verified in all cases. Retrogradely labeled neurons were seen in the paraventricular and dorsomedial hypothalamic nuclei and in the lateral hypothalamic area. Small groups of neurons projecting to one labeled spinal level were interspersed with small groups projecting to the second labeled level, but no double labeled neurons were seen unless the injections were placed into adjacent spinal levels. There was no simple topographic pattern to the retrograde labeling, but hypothalamic neurons appeared to project almost exclusively to single spinal levels.

These data indicate that, like the functionally specific preganglionic neuron pools of the medulla and spinal cord, the hypothalamic-autonomic cell pools are intermixed, but have anatomically distinct targets. This organizational principle appears to underlie the functional specificity of descending hypothalamic-autonomic projections and explains the difficulty of previous investigations in identifying an organizational pattern.

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22.2 **BRAINSTEM AREAS WHICH PROJECT TO CARDIAC REGIONS OF THE NUCLEUS AMBIGUUS IN RAT.** S.E. Fish, S.L. Stuesse, and K.S. Powell.* Neurobiology Program, N.E. Ohio College of Med., Rootstown, OH 44272.

The nucleus ambiguus (NA) is a brainstem structure which sends projections through the vagus nerve to the viscera, primarily heart, lung, and gut. The anatomical relationship among the NA and other brainstem structures has not been elucidated nor has the cardiac region been identified physiologically in rats. We have attempted to clarify which areas of the NA are cardioinhibitory and to determine other regions of the brainstem which send direct projections to this physiologically identified cardiac region. Stimulating electrodes were positioned sterotaxically in the medulla of anesthetized rats. Small currents (25-30ua/.2ms) were passed through the electrodes to locate regions in the ventrolateral medulla which slowed heart rate. In each rat, the area found was small (less than 200 um in diameter) very specific, and located in the most rostral portion of the NA just caudal to the facial motor nucleus. Microquantities of horseradish peroxidase (HRP Sigma type VI) were then iontophoretically ejected into this brainstem area. 24-72 hours following the HRP injection, the rats were processed for HRP reaction product using the tetramethybenzidine method. The only well-identified area from midbrain to upper cervical spinal cord which sent projections to the rostral NA was the ipsilateral medial subnucleus of the solitary tract (mnTS) HRP-filled cell bodies in the mnTS were found 1000um to 100um above the obex. A few labeled cells were found in the ipsilateral ventrolateral subnucleus of the solitary tract at the level of the obex and in the reticular formation. Control injections in reticular areas surrounding the rostral NA showed no label in the mnTS. These results are consistent with the interpretation that some of the mnTS cells project to cardioinhibitory regions of the NA. Since this NA area is also surrounded by cells with activity correlated with respiration, we can not rule out the possibility that some of these direct projections may subserve pulmonary functions. Supported by NIH HL23964 and a grant from the American Heart Association, Akron District Branch.

22.3 ASCENDING FASTIGIAL PROJECTIONS IN THE BEAGLE. R.J. Person, K. J. Dormer, and J. A. Andrezik. Depts. of Physiol. and Biophys. and of Anat. Sci., Univ. of Okla. HSC. Oklahoma City, OK 73190.

In the dog electrical stimulation of the cerebellar fastigial nucleus (FN) evokes a stimulus-bound pressor response (FPR) and baroreceptor-modulated bradycardia. Chronic stimulation in awake dogs also elicits a stimulus-bound stereotyped pattern of oral behavior of compulsive licking or grooming directed towards the forelimbs. Preliminary investigation of FN efferent pathways in the beagle was initiated using autoradiographic procedures. Beagles (10-12 kg) were anesthetized with α-chloralose (115 mg/kg); ECG electrodes and femoral artery cannulae were placed to record heart rate and blood pressure. Concentric electrodes were placed in the rostral FN and stimulation current (100-500 μA, 80 Hz) passed to localize the site eliciting maximal FPR and tachycardia. The electrode was replaced with a micropipette and a 1:3 mixture of ^{3}H-leucine and proline (60-120 μC/μl) injected in volumes of 30-120 nl. Dogs recovered for 10-14 days. The brain was processed for autoradiography using standard techniques. A description of the brain stem projections identified in these dogs was reported recently (Fed. Proc. 41:1517, 1982). Analysis of injection sites showed that significant numbers of ascending labeled axons were seen only with spread of protein label to middle and caudal FN. Injection material confined to the rostral FN resulted in labeling of fibers extending only to the substantia grisea centralis at the level of the oculomotor nucleus. With middle and caudal FN injections ascending labeled fibers were primarily contralateral to the injected FN. Fibers in transit were seen as a diffuse contingent ascending lateral to the borders of the substantia grisea centralis and oculomotor nucleus. Terminal areas were found in the deep layers of the superior colliculus bilaterally, the ipsilateral side receiving fibers decussating through superior collicular and posterior commissures. Passing the caudal pole of the thalamus the contingent splits ventral to the central medial thalamic nuclei and enters the thalamus with (1) a small decussating projection terminating diffusely in the ipsilateral ventromedial (VM) and ventrolateral (VL) nuclei, (2) a small dorsally-directed projection terminating in the contralateral medial dorsal nucleus, and (3) a larger projection to the contralateral VM and VL. Sparse labeling was observed in the centrolateral, paracentral, and central medial nuclei. It may be that the oral behavior directed to the forelimb may be an attempt to investigate or relieve odd sensation resulting from the convergence of FN and sensory projections on thalamic neurons involved in forelimb posture control. Supported by in part by USPHS grants HL 24082 and NS 16868.

22.4 PATHWAYS FROM THE FASTIGIAL PRESSOR AREA IN RAT. A. Del Bo, D.A. Ruggiero, C.A. Ross, R. Wiley and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

We sought to map projections from neurons of the rostral fastigial nucleus (FN) at sites from which electrical stimulation elicits a fastigial pressor response (Miura and Reis, Brain Res. 13, 595, 1969). Anterograde tracers, horseradish peroxidase-wheat germ agglutinin and ^{3}H-amino acids were microinjected into pressor sites of rostral FN in rats anesthetized with halothane and identified by the TMB method and autoradiography, respectively. With injections that selectively involved cells in rostral FN, anterograde transport was confined to the hindbrain and distributed primarily via two trajectories: a) the contralateral uncinate fasciculus (UF) and, b) the ipsilateral juxtarestiform body (JRB). A few additional fibers projected into the ipsilateral UF, superior medullary velum and cerebellar vermis.

The UF projections decussate within the cerebellum arch rostrally over the contralateral dorsal parabrachial n. and caudally over the dorsal convexity of the superior cerebellar peduncle. The UF passes ventrally along the lateral border of the superior vestibular n. (SVN), arches medially and traverses a ventral strip of the rostral lateral vestibular n. (LVN). At the level of SVN-LVN fibers of the UF follow two courses: 1) One group splits into a series of descending fascicles, which lie between SVN-LVN and the inferior cerebellar peduncle, are traversed by entering rootlets of the vestibular nerve, and descend caudally throughout lateral aspects of the entire vestibular complex. These give rise to a thin medially directed fiber sheet primarily traversing and terminating in ventral parts of inferior (IVN), and medial (MVN) vestibular n., perihypoglossal structures, and throughout n. parasolitarius (NPS). 2) The second group terminates throughout ventral LVN and also gives rise to fibers which leave LVN and spray ventromedially into the dorsal pontine tegmentum to terminate primarily within specific subdivisions of gigantocellular, paramedian and lateral reticular n., and n. ventralis.

The fastigial contingent of the JRB is ipsilateral and traverses SVN and LVN. It contributes to the terminal fields of the UF within LVN and IVN, but does not appear to enter the reticular formation.

Thus, the effects of FN stimulation upon blood pressure, release of vasopressin (Del Bo et al., Fed. Proc., 1982) or cerebral blood flow (Nakai et al. Brain Res., 1982) are either mediated by polysynaptic pathways or by excitation of fibers passing through the rostral FN. Projections from neurons in pressor sites of FN do not extend rostral to the hindbrain, nor descend to autonomic segments of spinal cord. The autonomic responses, therefore, may be subserved by polysynaptic pathways synapsing in hindbrain. Another possibility is that stimulation of the rostral FN excites fibers of passage originating from other regions of cerebellum.

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22.5 INSULAR AND MEDIAL FRONTAL CORTEX PROJECTIONS TO THE AMYGDALOID CENTRAL NUCLEUS IN THE RABBIT. B. S. Kapp, J. S. Schwaber[1], and P. A. Driscoll-Mendes*. Dept. of Psychology, The Univ. of Vermont, Burlington, VT 05405, and [1]Dupont Central Research Neurobiology Group, Glenolden, PA 19036.

Recent evidence in the rabbit has demonstrated that (1) the amygdaloid central nucleus projects directly to the nucleus tractus solitarius and the dorsal motor nucleus of the vagus nerve (Schwaber et al., 1980, 1982), (2) electrical stimulation of the central nucleus elicits bradycardia and depressor responses (Kapp et al., 1982), and (3) lesions or pharmacological manipulations within the central nucleus attenuate vagally-mediated conditioned bradycardia (Kapp et al., 1979; Gallagher et al., 1980, 1981). These data suggest a role for the central nucleus in cardiovascular regulation. As one of a series of studies designed to further elucidate the nature of this role, we are investigating the afferentation of this nucleus in the rabbit. The present study examined central nucleus projections originating in the frontal cortex.

Twenty-two rabbits received pressure injections of HRP (25-50%; 10-50nl) or the retrograde fluorescent tracers, Bisbenzimide (5-10%; 10-60nl) or Nuclear Yellow (5%; 15nl), delivered via a glass micropipette (40-60 μm tip diameter) or 26 gauge syringe needle and aimed at the central nucleus. Animals were sacrificed from six hours to three days following the injection. The HRP-injected brains were processed using the TMB procedure (Mesulam, 1978), while those injected with BB and NY were examined using fluorescence microscopy.

Central nucleus injections produced significant numbers of labeled neurons located in the lateral insular and medial frontal cortex. Those located in the insular cortex were primarily restricted to layer V of the agranular insular cortex at more rostral levels. At more posterior levels the labeled neurons were located in layers II-III as well as layer V and extended dorsally into layer V of the ventral portions of the granular insular cortex. Injections of HRP into the insular cortex resulted in anterograde labeling within the central nucleus, thereby confirming the direct cortico-central nucleus projection. Labeled neurons located in the medial frontal cortex were located primarily in layer V of the prelimbic cortex (Area 32) and were scattered throughout layers II-V of the indistinctly laminated infralimbic cortex (Area 25). While the exact terminal distribution of these cortical projections within the central nucleus has yet to be determined, the present results nevertheless demonstrate a significant cortical influence on the amygdala central nucleus and possibly on those neurons which project to medullary cardiovascular regulatory nuclei. Supported by PHS NS 16107.

22.6 DIRECT PROJECTIONS TO AUTONOMIC CENTERS OF FOREBRAIN AND BRAINSTEM FROM A CORTICAL VASOPRESSOR AREA IN RAT. S. Mraovitch, D.A. Ruggiero, C.A. Ross and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

A region of nucleus tractus solitarius (NTS) upon which cardiopulmonary afferents terminate (Ross et al. Brain Res. 223, 402, 1981) is innervated by a major projection from insular cortex (areas 13-14 of Krieg, 1946). In the present study, we sought to a) determine whether electrical stimulation of this region elicits cardiovascular responses, and b) trace anatomical pathways from active sites. Electrical stimulation of insular cortex (8 sec train, 100 μA, 50 Hz, 0.5 msec pulse duration) in anesthetized (60 mg/kg chloralose) paralyzed rats (n=5) increased arterial pressure (AP) and heart rate (HR). At active sites, threshold current was 50 μA. At optimal frequencies (50-300 Hz), and at 100 μA cortical stimulation increased AP $\leq$ 50 mm Hg and HR $\leq$ 40 bpm. Most responsive sites were concentrated between claustrum and rhinal fissure and corresponded to the insular area of Krieg. Wheat germ agglutinin-horseradish peroxidase was injected into areas corresponding to active sites, and sections processed with the TMB method. Anterogradely labeled fibers were mapped. Projections from cortex were distributed throughout brain, particularly in thalamocortical and limbic-autonomic structures. In forebrain, major unilateral projections end in n. stria terminalis, central amygdala n., lateral pallidal segment, ventral striatum. Less intense projections end in lateral and basal amygdala n., medial pallidal complex and substantia innominata. In thalamus, major projections end primarily in ventrobasal-ventromedial n., intralaminar-midline n., medial-dorsal and medial geniculate n. There were relatively weak terminals in hypothalamus, with minor projections to zona incerta, supra- and tuberomammillary n., and lateral hypothalamic fields. In midbrain and hindbrain, corticobulbar fibers heavily innervate autonomic regions of central grey, Edinger-Westphal n., parabrachial and solitary tract n., A5 area and ventrolateral medulla. Other projections were to substantia nigra, precerebellar and trigeminal n. There were no projections to spinal cord. In NTS, terminals lie primarily in gustatory zones and also overlap the baroreceptor-vagal innervation. Retrograde transport data indicate that cortical cells projecting to NTS-cardiopulmonary subdivisions are topographically distinct — lying ventral to and in a more restricted location from the origin of projections to reticular formation and gustatory NTS. Cortical control of the autonomic nervous system has traditionally been viewed as mediated by hypothalamic and corticospinal tracts. However, these results demonstrate the presence of numerous direct projections from autonomic cortex to other widely distributed autonomic areas throughout brain. These direct cortico-autonomic pathways may regulate circulatory and other autonomic functions.
(Supported by NIH Grant NS 03346.)

22.7 IDENTIFICATION OF NEURONAL POPULATIONS PROJECTING FROM THE VENTRAL MEDULLA TO OTHER NUCLEI WHICH REGULATE AUTONOMIC FUNCTIONS. J.A. Andrezik, S.R. Holmes,* S.R. Anderson* and M.W. Halterman,* Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

The nucleus paragigantocellularis lateralis (PGCL) and the nucleus gigantocellularis, pars alpha, (GCα) project to the intermediolateral cell column of the rostral thoracic spinal cord and to other nuclei which influence the autonomic nervous system (Loewy et al., Br. Res. Rev. 3:63-80, 1981). The diverse connections of these reticular nuclei and their hetrogeneous cell populations (Andrezik et al., Anat. Embryol. 161:355-371, 1981) suggested further studies be made as to which neurons of the ventral medulla (VM) project to other autonomic nuclei and as to whether or not the same neuron sends axons to more than one nucleus.

Sprague-Dawley rats were injected with the fluorescent dye Fast Blue (FB), and one of the dyes Nuclear yellow (NY) or Bisbenzimide (BB). The injections (30-60 nl) were asymmetrical and in terminal areas of VM axons, i.e. the parabrachial nucleus (NPB), dorsal medulla (DM, including the solitary and dorsal vagal nuclei), paraventricular nucleus of the hypothalamus (PVH), periaqueductal gray (PAG), zona incerta (ZI), and intermediolateral nucleus of the thoracic spinal cord (IML). These sites were used in a variety of combinations to form pairs which were analyzed for double-labeling and for cell populations.

Neurons projecting to NPB are found bilaterally but primarily ipsilateral in the caudal PGCL; medium-sized and large triangular neurons, and some large round neurons are labeled. PVH injections result in only a few neurons labeling in the ipsilateral PGCL, while ZI injections label neurons mainly in the contralateral dorsal PGCL. These neurons are separate populations from those that project to NPB. With PAG injections the labeling is bilateral and confined to the medial PGCL and lateral GCα caudal to the facial motor nucleus. Spindle-shaped cells, as well as medium-sized and most of the large triangular neurons are labeled. Injections into DM label neurons in the caudal PGCL of both sides. Projections to the IML and spinal cord below the rostral thoracic segments originate in the ipsilateral GCα; only a few cells in the ventral PGCL contribute to this pathway.

For the most part, individual populations of neurons in VM project to separate "autonomic nuclei." In all our cases only about 10% of all labeled neurons contain both dyes; the majority of the double-labeled neurons project to PAG and DM. (Supported by USPHS grant NS-16868.)

22.8 PREGANGLIONIC NEURONS AND VISCERAL AFFERENT FIBERS IN THE RAT PELVIC NERVE. I. Nadelhaft and A.M. Booth. VA Medical Center & Depts. of Neurosurgery and Pharmacology, University of Pittsburgh, School of Medicine, Pittsburgh, PA.

The location and morphology of preganglionic neurons of the sacral parasympathetic nucleus (SPN) and the central distribution and organization of related visceral afferent fibers running in the rat pelvic nerve was determined using the horseradish peroxidase (HRP) tracing technique. HRP was applied to the central portion of the transected pelvic nerve of adult female Sprague-Dawley rats (about 450 gm) which were sacrificed two days later. The SPN was located ipsilaterally in S1 and L6 and formed a continuous column of about 850 cells extending 3.7mm rostrocaudally. In transverse sections, cells were found in a pocket about 100μm in diameter along the lateral gray-white border at the base of the dorsal horn. Cells were round, triangular and spindle shaped (average size: 11x20μm) and were oriented in all directions. Dendrites extended into the dorsolateral funiculus, the lateral marginal zone of the dorsal horn, and horizontally into the dorsal gray commissure. Axons ran along the lateral gray-white border.

Afferent fibers transported HRP to the ipsilateral dorsal root ganglia (DRG) and from there to the spinal cord. Labelled cells (average size: 19x29μm) were found in S1 and L6 DRGs in numbers proportional to the numbers of SPN neurons found in corresponding cord segments. Centrally, afferent fibers entered Lissauer's tract (LT) via the S1 and L6 dorsal roots and were observed in LT rostrally as far as L2, and caudally to S3. Afferent collateral fibers from LT formed two groups: a prominent lateral projection located along the lateral margin of the dorsal horn and which entered the SPN region, a less prominent medial projection extending along the dorsal margin of the dorsal horn and alongside the dorsal columns to the dorsal gray commissure. When observed in horizontal sections, these collateral groups were seen to be organized into periodically spaced fiber bundles (average center to center distance: ∿100μm).

An unexpected bundle of afferent fibers was observed just below the ventral border of the central canal. It was 10μm in diameter and was observed to run continuously from S3 to L2. The source of these fibers was not determined.

In summary, with the exception of this unusual afferent bundle, the organization of pelvic nerve afferents and efferents in the rat paralleled the results of similar studies in cats and monkeys. Features in common were: 1) the location of the SPN, the number of preganglionic neurons and their dendritic patterns, 2) the division of afferent collaterals into strong lateral and weaker medial projections and the periodic organization of these collaterals, 3) the relationships among collateral projections, preganglionic neurons, and dendrites.

22.9 THE ORIGIN OF RENAL SYMPATHETIC EFFERENTS, AND THE CENTRAL PROJECTION OF RENAL AFFERENTS IN CAT. D.C.Kuo, W.C.deGroat, I. Nadelhaft, T.Hisamitsu*, and M.G.Backes*. Dept.of Pharmacology, Univ. of Pittsburgh, Sch. of Medicine and VA Medical Center, Pittsburgh, PA 15261

The cells of origin of renal efferents, and the segmental distribution and central projection of renal afferents in adult cats and kittens were studied by the technique of retrograde and anterograde transport of horseradish peroxidase (HRP). Nerves on the surface of the left renal blood vessels were transected near the hilum of the kidney, and the central cut ends exposed to HRP. Sympathetic efferent neurons were labeled centrally along the renal nerve, in the superior mesenteric ganglion, and in the ipsilateral sympathetic chain ganglia (T12-L3). HRP was not detected in preganglionic neurons in adult animals but a few of these cells were labeled in the spinal cord of kittens. The results show that the sympathetic efferent innervation of the kidney is derived from both paravertebral and prevertebral ganlia. In the latter (superior mesenteric ganglion), renal afferent neurons exhibited a topographic distribution. Labeled cells were localized in the caudal pole of the ganglion near the origin of the renal nerves.

Retrograde transport of HRP labeled renal afferent perikarya in the ipsilateral dorsal root ganglia (DRG) from T12 to L4. A few labeled cells were also discovered in contralateral DRG. Transganglionic anterograde transport of HRP successfully labeled renal nerve afferent fibers (RNAF) in the spinal cord of kittens. RNAF were found between T11-L6, with the greatest concentration in L1, L2, and L3. Fibers traveled rostrocaudally in medial and lateral Lissauer's tract; some collaterals could be traced into and through lamina I. In the transverse plane, a medial (MP) and a lateral (LP) renal projection within the dorsal horn margins could be readily discerned. A large number of fibers in LP passed medially into Lamina V at the base of the dorsal horn. In some sections LP could be followed ventrally into the lateral portion of upper lamina VII where the intermediolateral nucleus is located. Fibers in MP and LP could also be traced across the midline in the dorsal commissure. In the contralateral spinal cord RNAF were largely seen in Lamina V. These results demonstrate that RNAF project into regions containing spinothalamic tract cells, and therefore may reflect sites of termination of renal nociceptive pathways. These afferents as well as other projections into autonomic nuclei may be involved in spinal sympathetic reflexes to the kidney. Supported in part by NSF Grant PCMF9O6093, Clinical Research Grant MH30915, NIMH Grant 5430, NIH Grant 1-F32-NSO7006, National Spinal Cord Injury Foundation, and Western Pennsylvania Heart Association.

22.10 SPINAL ORGANIZATION OF RESPIRATORY INFLUENCES ON SYMPATHETIC NERVE DISCHARGE PATTERNS IN CATS. C. A. Connelly*, R. D. Wurster. Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153.

Sympathetic nerve discharges exhibit respiratory rhythmicity. Descending pathways which alter sympathetic activity originate in the brainstem and are located primarily in the dorsal and ventrolateral regions of the spinal cord. Those fibers affecting phrenic and intercostal motor nerves are located in the ventrolateral spinal cord. The purpose of this study was to 1) determine if fibers responsible for the respiratory rhythmicity in sympathetic nerves are also localized in the ventrolateral spinal cord and 2) determine the laterality of descending respiratory influences. Inferior cardiac sympathetic, phrenic and intercostal nerve activities were recorded and integrated in vagotomized, alpha-chloralose anesthetized cats which were paralyzed and artificially ventilated. Respiratory rhythmicity was assessed by comparing the sympathetic discharge with phrenic nerve activity. Intercostal nerve activity served to assess the lesion of descending spinal respiratory pathways. Progresive lesions in the C6 and C7 segments of the spinal cord were made below the C5 level of phrenic motor outflow. The following results were obtained: 1) Bilateral lesions of the ventral cord interrupted the respiratory activity of the intercostal nerve while the phrenic and sympathetic nerves remained in phase. 2) Ipsilateral hemisections attenuated but did not eliminate the respiratory rhythm in the inferior cardiac sympathetic nerve discharge. 3) Bilateral dorsal spinal cord section was necessary for complete elimination of the respiratory rhythm in sympathetic nerves. It is concluded that the rhythmic nature of sympathetic discharges is primarily due to bilaterally descending influences located in the dorsal spinal cord. (Supported by NIH Grant HL 08682 and The Potts Foundation.)

22.11 VAGAL AND GASTRIC CONNECTIONS TO THE CENTRAL NERVOUS SYSTEM DETERMINED BY THE TRANSPORT OF HORSERADISH PEROXIDASE. F. C. Barone, S. L. Scharoun, B. B. Falk,* P. A. McGrattan* and M. J. Wayner. Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210.

Pure horseradish peroxidase (HRP, Sigma Type VI) crystals were encased in a parafilm envelope and applied to the transected central ends of the left and right cervical vagus nerves and the anterior and posterior esophogeal vagus nerves of adult male hooded rats. Injections of HRP (30%) were made into the muscle wall of the fundus and antrum regions of the stomach. After a 48 hr survival time, animals were perfused intracardially with a phosphate buffer plus sucrose wash followed by glutaraldehyde and paraformaldehyde fixative. The brain stem, spinal cord segments and corresponding dorsal root ganglia, the superior cervical sympathetic ganglion, and the nodose ganglion 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 Mesulum . Sequential sections were examined under a microscope. Labeled neurons and nerve terminals were identified using bright and dark field condensers and polarized light. In tissue from animals that had HRP applied to the cervical vagus nerves, retrogradely labeled neurons were identified ipsilaterally in the medulla located in the dorsal motor nucleus of the vagus (DMN) and in the nucleus ambiguus (NA). Labeled cells extended from the DMN into the spinal cord in laminae X of cervical segments. Many bipolar neurons were labeled in the nodose ganglion. Anterogradely labeled terminals were observed throughout and adjacent to the solitary nucleus (SOL) dorsal to the DMN and intermixed among labeled neurons located in the DMN. In tissue from animals that had HRP applied to the esophogeal vagus nerves, similar labeling was observed. However, fewer neurons were identified in the NA, the nodose ganglion, and in the cervical spinal cord. Also, very little terminal labeling was observed in and adjacent to the SOL. Labeled neurons in tissue from animals that had HRP injected into the stomach wall were observed bilaterally in the DMN, nodose ganglion, and only at the C_1 level of the spinal cord. However, labeled bipolar neurons were observed in the dorsal root ganglia of the thoracic cord. These data indicate that cervical cord and NA neurons are more important in the supradiaphragmatic motor innervation by the vagus. Also, many afferents to the SOL originate above the diaphragm. In addition, some afferents from the stomach enter the central nervous system via the thoracic spinal cord.

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22.12 SKIN RESISTANCE LEVEL IDENTIFIES LATERALIZED AUTONOMIC DEFICIT. R.L. Bruno*, L.J Cote and J.A. Downey*. Research Labs., Dept. of Rehabilitation Med., College of Physicians and Surgeons, Columbia Univ., N.Y., N.Y. 10032.

Autonomic dysfunction is often seen in patients with Parkinson's Disease (PD). For example, patients frequently exhibit diminished sweating on the trunk and limbs (Appenzeller and Goss, 1971). However, it is unclear whether the diminution in sympathetic cholinergic activity which underlies such anhydrosis is attributable to peripheral neuropathic changes (which have been demonstrated in PD patients (Aminoff and Wilcox, 1971)) or to the central degenerative process which underlies the disease.

The skin resistance level (SRL) is a sensitive measure of the degree of hydration of the stratum corneum of the skin, and thus of sympathetic cholinergic efferent activity (Edelberg, 1972). The greater the sympathetic cholinergic outflow to the sweat glands and corneum, the greater the hydration of the skin and the lower the SRL. The SRL of both hands was recorded at rest in 18 dextral PD patients who had asymmetric symptoms, i.e. their overall symptoms and their upper extremity tremor or rigidity were greater on one side of the body. The patients separated equally into those with predominant left- or right-sided involvement; they were receiving anticholinergic agents and/or L-Dopa at the time of testing. It was found that the mean SRL was 46.5% higher in the hand on the more involved side of the body ($d=239$ Kohms; $t=2.06$; $p<.05$) and that the SRL of the right hand was significantly correlated with the severity of the rigidity in that hand ($r=.702$; $p<.05$).

The higher SRL on the more affected side of the body and the correlation between SRL and rigidity demonstrate a diminution in sympathetic cholinergic activity in these patients, the magnitude of which is related to the distribution and severity of the PD symptoms. These SRL findings strongly suggest that the sympathetic cholinergic deficit in PD is attributable to a central autonomic disorder related to the motor symptoms, rather than to degenerative changes in the peripheral autonomic nervous system.

22.13 EFFECTS OF FOOD DEPRIVATION ON LIVER AND HEART ADRENERGIC RECEPTOR MECHANISMS DURING ONTOGENY. C.M. Kuhn, M.K. McMillian*, G.E. Evoniuk* and S.M. Schanberg. Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.

We have reported previously that in preweanling rat pups food deprivation (FD) causes a selective loss of liver responsivity to alpha adrenergic agonists. We now report that FD affects both liver and heart responsivity to adrenergic stimuli but that the mechanism responsible for altered responsivity changes during ontogeny. Short term FD (2 hrs) of 10 day old rat pups decreases liver ornithine decarboxylase (ODC) responsivity to both the alpha adrenergic agonist phenylephrine (PHE) and the beta adrenergic agonist isoproterenol (ISO). However, the number of hepatic $alpha_1$ and beta receptors as determined by measuring the binding of ^{3}H-prazosin and ^{125}I-pindolol to crude membrane preparations is not changed. In addition, heart ODC responses to PHE and ISO are not affected by short term FD. Long term FD (12-24 hrs) of 10 day old pups also is associated with decreased liver responsivity to PHE and ISO that is dissociated from changes in receptor number: beta receptor number actually increases although responsivity is decreased, and $alpha_1$ receptors do not change.

In adults, short term FD affects neither liver ODC responses to adrenergic agonists nor receptor number. However, long term FD (24-48 hrs) causes an increase in beta receptor number and a decrease in $alpha_1$ receptor number which are accompanied by a parallel increase in liver ODC responsivity to ISO and a decrease in responsivity to PHE. Heart responsivity to both PHE and ISO is decreased following long term FD of adult rats, and the decrease in responsivity to these adrenergic agonists is accompanied by a decrease in both $alpha_1$ and beta receptor number.

These results suggest that nutrition plays an important role in regulation of peripheral adrenergic receptor mechanisms. However, the duration of FD required to change responsivity and the mechanism by which the changes occur are different in neonates and adults. The effects of FD in neonates occur after only 2 hrs of FD, and seem to be mediated by postreceptor mechanisms, while altered responsivity in adults only occurs after 24-48 hrs of FD, and altered responsivity can be explained by changes at the receptor level. Finally, FD has opposite effects on beta receptor mechanisms in liver and heart: beta responses in liver are potentiated by FD, while those in heart are suppressed.

22.14 STREPTOZOTOCIN DIABETES-INDUCED CHANGES IN AUTONOMIC RECEPTOR SENSITIVITY IN THE RAT BLADDER. Michael C. Gerald and Malak G. Kolta.* Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210.

Urinary bladder dysfunction is a chronic complication of diabetes mellitus. This autonomic neuropathy is characterized by progressive bladder paralysis with urinary retention. The present study was designed to test the hypothesis that diabetes alters the sensitivity of the detrusor and sphincter + trigone (Sph+T) smooth muscles of the bladder to cholinergic and/or adrenergic (α,β) activation, autonomic influences that normally control bladder function.

Diabetes was induced with streptozotocin (40 mg/kg, i.v.) in adult, male Sprague-Dawley rats and tissue sensitivity to autonomic drugs was assessed 6 wks later. Average plasma glucose levels and percent changes in initial body weight in control and diabetic rats were 104±3 and 497±23 mg %, and +44% and -5%, respectively. At the time of sacrifice, visual inspection revealed that bladders obtained from diabetic rats were much more distended than controls. Preliminary studies indicated that, while the contractile response of control and diabetic intact bladders to KCl was equivalent, diabetic bladders were 2-fold more sensitive to acetylcholine (ACh). Subsequent studies compared the sensitivity of the detrusor and Sph+T smooth muscles from control and diabetic rats to KCl and the cumulative addition of: ACh, phenylephrine in propranolol-pretreated tissues, and isoproterenol in phentolamine-pretreated tissues. Results were analyzed as a percentage of the maximal response to KCl. Control and diabetic tissue sensitivity to KCl was equivalent. Although ACh produces relaxation in the human Sph+T, contraction was observed in rats. Diabetic detrusor and Sph+T muscles were 4- and 100-fold more sensitive, respectively, to ACh-induced contractions. The maximum contractile response to phenylephrine (α-adrenoceptor agonist) was 2.5-fold greater in the diabetic detrusor, while no difference was observed in the Sph+T. While the diabetic detrusor was more sensitive to isoproterenol-induced relaxation, the diabetic Sph+T was less responsive to relaxation by this β-agonist than corresponding controls.

These results suggest that the diabetic state induces selective alterations in cholinergic and/or adrenergic receptor sensitivity and support the hypothesis that diabetic urinary bladder dysfunction may result from β-mediated enhanced detrusor relaxation and/or from a marked enhancement of the sensitivity of the Sph+T to cholinergic contraction.

22.15 Effects Of Glucose And Osmotic Pressure On Temperature-Sensitive Neurons In Hypothalamic Tissue Slices. N.L. Silva* and J.A. Boulant. Department of Physiology, Ohio State Univ., Columbus, Ohio 43210.

Previous studies have demonstrated the existence of temperature-sensitive single units in the preoptic area and anterior hypothalamus (PO/AH) in both *in vivo* and *in vitro* preparations. Nearly one half of the PO/AH neurons may be classified as temperature-sensitive; i.e., warm-sensitive cells increase their firing rates with increasing preoptic temperature; and cold-sensitive cells increase their firing rates with decreasing preoptic temperature. Other studies have suggested that certain PO/AH units may respond to alterations in either glucose or osmotic pressure. The purpose of the present study was to characterize the specificity of *in vitro* PO/AH neurons for their responses to temperature, low glucose and hyperosmolarity.

PO/AH tissue slices (300-400 μm thick) were maintained in a humidified chamber which was constantly perfused with an oxygenated nutrient media (7.4 pH, 300 mOsm/kg, 10 mM glucose). The medium perfusing the slices was maintained at 37°C, but could be rapidly changed between 32°-42°C. Low glucose media (1-3 mM glucose) or hyperosmotic media (307-309 mOsm/kg) could also be switched to perfuse the chamber. Following the determination of a single unit's extracellular response to changes in temperature (impulses/second/°C) the normal media was replaced by either the low glucose or hyperosmotic media. The spontaneous firing rate and temperature sensitivity were again examined during these experimental perfusions.

The primary effect seen in the cold-sensitive cells was an inhibition produced by the low glucose and hyperosmotic media. Within the temperature-insensitive cell group the majority of units were not affected by either media, however, 30% of these units were inhibited by either one or both media. Of the warm-sensitive cells studied, approximately 10% were facilitated by one or both media; and the remaining warm-sensitive neurons were not affected by either media.

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22.16 DELAY AND PARTIAL REVERSAL OF NEUROENDOCRINE REPRODUCTIVE DYSFUNCTION BY OVARIECTOMY IN MIDDLE-AGED AND OLD C57BL/6J FEMALE MICE. C. Mobbs*, D. Gee* and C. E. Finch. Andrus Gerontology Ctr., Univ. of So. Cal., Los Angeles, CA, 90007.

Reproductive senescence in C57BL/6J female mice 12 to 18 months of age is characterized by cessation of ovarian cycles and onset of persistent vaginal cornification (PVC). Mice with PVC have moderately elevated and sustained plasma estradiol (E2), show major impairments in the E2-induced LH surge ($p<.001$), and can sustain fewer estrous cycles when given young ovarian grafts ($p<.001$), as compared to 6-month-old cycling mice. Middle-aged (12 months) but still cycling mice also show these impairments, although to a less extent ($p<.05$). If 18-month-old mice were ovariectomized (OVXed) two months previously, after the onset of acyclicity and correlated neuroendocrine dysfunctions, the E2-induced LH surge and the ability to support cycles given young ovaries were both partially restored ($p<.01$) (see table below). Furthermore, if the 18-month-old mice had been OVXed 12 months earlier, while still young and cycling, there was no significant loss of function in the regulation of LH. However, if middle-aged but still cycling mice were OVXed for 2 months, there was no significant restoration of function in either regulation of LH or ability to support cycles.

Age[a]	Time OVXed[b]	LH(ng/ml) (n)	# cycles[c] (n)
6 mo.	4 days	200±45 (13)	17±1.6 (18)
6 mo.	2 mo.	300±50 (16)	....
12 mo.	4 days	90±20 (16)	7.1±3.1 (16)
12 mo.	2 mo.	120±30 (15)	5.9±2.6 (17)
18 mo.	4 days	20±5 (18)	1.6±0.8 (18)
18 mo.	2 mo.	120±30 (20)	7.6±1.2 (17)
18 mo.	12 mo.	190±50 (16)	....

[a]Age at grafting of young ovaries or induction of LH surge.
[b]Length of time OVXed before grafting of young ovaries or induction of LH surge.
[c]Total number of estrous cycles of host after ovaries were removed and replaced with 6-month-old ovarian grafts.

We conclude that ovariectomy of young cycling mice can prevent or delay loss of neuroendocrine reproductive function as they age; furthermore, 2 months of OVX in 18-month-old mice can partially restore reproductive function. However, OVX does not seem to affect the neuroendocrine dysfunctions of middle-aged mice. These data indicate that there may be a biphasic ovary-dependent component to reproductive senescence.

22.17 THE EFFECT OF INTRACRANIAL SELF-STIMULATION (ISS) ON THE RESPONSE TO COLD STRESS AMONG AGED MICE. M. Talan* (Spon.: N. Buckholtz) Gerontology Research Center, National Institute on Aging, Baltimore, MD, 21224.

Informal observation in my former laboratory (Pavlovian Institute, Leningrad, USSR) suggested that animals which were allowed to electrically self-stimulate their brains in so called reward areas also appeared more vigorous in settings outside of the test chamber. In order to test this impression further, the following study was carried out. In experiment 1 electrodes were implanted in the medial forebrain bundle in 30, C57BL/6J mice aged 30 mos. All animals were able to produce ISS after a short shaping procedure. They were divided in two groups of 15 mice each. After 10 days all animals were exposed to a 10°C cold stress for 3 hr while rectal temperature was recorded every 30 min. After three weeks all animals were retested. Between the two tests animals in the first group (+ISS) received daily sessions of ISS for 30 min. Animals in the second group (-ISS) were placed in the ISS chamber every other day for 30 min but were not allowed ISS. Data were analyzed by computing individual slopes of rectal temperature during the cold stress for each test. These regression coefficients then were compared using a repeated measures analysis of variance. The results showed that there was no difference between groups of test 1, F (1,28) =.00058, but at test 2 the -ISS group had significantly poorer tolerance to the cold stress than the +ISS group, F (1,28) = 20.16, p <.001. This difference occurred because cold tolerance in the -ISS group fell significantly from test 1 to test 2, F (1,28) =23.37, p<.001; whereas the +ISS group showed no deterioration in performance, F (1,28) =.102.

In experiment 2 electrodes were implanted in 6, 30 mo. old mice which were tested for tolerance to cold stress as described above. Then they were retested twice at 3 week intervals. Between test 1 and test 2 they remained in their home cages; between test 2 and test 3 they received 30 min/day sessions of ISS for 3 weeks. The results showed a significant test effect, F (2,10) =9,23, p < .01, body temperature fell significantly more during test 2 than test 1, F (1,10) =9.19, p <.01. However, during test 3 the temperature response to cold stress was not different from test 1, F (1,10) = 1.3, but was significantly better than during test 2, F (1,10) = 17.42, p<.01.

These data suggest that ISS may facilitate the ability of old mice to cope with cold stress.

23.1 EFFECTS OF LEAD ADMINISTRATION ON POSTNATAL DAY 5 OR DAY 15 OF LIFE ON LITHIUM-INDUCED POLYDIPSIA, STRIATAL ^{3}H-SPIPERONE BINDING AND DOPAMINE METABOLISM IN ADULT RATS. R.B. Mailman, D.L. DeHaven and M.R. Krigman. Biol. Sci. Res. Ctr. and Depts. of Pharmacol., Psychiat. and Pathol., Univ. North Carolina Sch. Med., Chapel Hill, NC 27514

Lithium-induced polydipsia (LIP) has been shown to be increased in adult rats who were treated with lead (Pb) from day 3 to day 30 of life (Mailman et al., Science, 201:637, 1978). This effect is permanent, is not caused by higher doses of lead administered from days 30-60 of life and is not due to differences in circulating renin or angiotensin or other renal changes. Lithium-induced polydipsia has also been shown to be dependent upon an intact nigrostriatal dopaminergic system, but not any other monoamine system. The present experiments have narrowed the range of ages during which Long-Evans rats are sensitive to lead-induced changes in LIP, and have also examined the function of the nigrostriatal dopamine system. In the first study, rats were treated by oral gavage with 200 mg/kg/day of lead (as the acetate) or with sodium acetate vehicle during days 3-10, 11-20 or 21-30 of life (birth = day 1). At 60 days of age, testing for LIP was initiated. Significant increases in LIP were seen in the groups treated from days 3-10 and 11-20, but not those treated on days 21-30. In the second experiment, rats were treated with a single dose of lead (200 mg/kg) or vehicle on either day 5 or day 15. A within-litter design was used such that each sex within a litter received one of the four treatments. Testing for LIP began when the rats were ca. 60 days of age, and the same rats were sacrificed for neurochemical analyses when ca. 6 months of age. Individual Scatchard analyses of ^{3}H-spiperone binding were performed for each rat's striata using unlabeled spiperone as displacer. Concentrations of dopamine, DOPAC and HVA were quantified using HPLC with EC detection (Kilts et al., J. Chromatog., 225:337, 1981). Administration of Pb on either day 5 or day 15 caused a significant increase in LIP compared to controls. However, when the animals were sacrificed after thorough washout of lithium, there was no difference between Pb-treated and control groups in the affinity or number of receptors for ^{3}H-spiperone in the striatum. Moreover, concentrations of DA, DOPAC and HVA in striatum were also unchanged. These data indicate the gross alterations of nigrostriatal DA function are not observable in treated rats who have significant alterations of LIP. However, differences between Pb and control groups might be evident if these same parameters were compared while the rats were being treated with lithium. In any event, the fact that a single dose of 200 mg/kg of lead causes a change in LIP of similar magnitude to that induced by dosing from days 3-30 suggests that this effect is a sensitive monitor of lead exposure during development. (Supported in part by ES-01104, ES-02087 and HD-03110).

23.2 EVIDENCE THAT LOW-LEVEL DEVELOPMENTAL LEAD EXPOSURE PRODUCES TOXIC AMBLYOPIA. D.A. Fox and A.A. Wright*. Div. of Toxicology, Univ. of Texas Med. Schl., Houston, TX 77025.

Clinical reports of children poisoned by inorganic lead (Pb) describe irreversible impairments in spatial resolution properties of the visual system. Experimental work from our laboratory and that of others suggests that deficits are confined to higher spatial frequencies and preferentially occur at scotopic, in contrast to photopic, levels of luminance. These changes have been reported to occur at all levels of the visual system (Fox et al., Tox. Appl. Tox.40:449,1977 and Neurobehav. Tox.1:101,1979; Fox and Sillman, Science 206:78,1979; Bushnell et al., Science 196:333, 1977; Impelman et al., Neurosci. Abs.8:in press,1982). To determine if the spatial resolution limit, visual acuity, of the adult rat's (100 days of age) visual system was altered following low-level neonatal Pb exposure, electrophysiological and psychophysical studies were conducted at mesopic and scotopic levels of luminance. Female neonatal Long-Evans hooded rats were exposed to Pb (or no Pb) from parturition to weaning (days 0-21) via the milk of dams consuming 0.2% Pb acetate (or water) solutions. Checkerboard pattern-reversal cortical evoked potentials (temporal freq. 0.94 Hz) were recorded in chronically implanted awake rats and used to estimate visual acuity by a modification of the extrapolation technique (Campbell and Maffei, J. Physiol.207:635,1970). Visual acuity psychometric functions were obtained utilizing sine-wave gratings (0.1-1.3 c/deg) in a conditioned suppression paradigm.

Results in control and Pb-exposed rats reveal a close correspondence between electrophysiologically and psychophysically determined spatial resolution limit. The maximal spatial resolution in control rats was approx. 1.3 c/deg under scotopic and mesopic conditions. These acuity values are in good agreement with those of Birch and Jacobs (Vis. Res.19:933,1979) and Lennie and Perry (J. Physiol.315:69,1981). Pb-exposed rats exhibited a 25-50% decrease in visual acuity with greater deficits being observed under scotopic, than mesopic, conditions. Decreases in spatial resolution were observed at all spatial frequencies greater than 0.25 c/deg in Pb-exposed rats. In addition to the decreased P2N2 amplitudes, which account for the decrements in spatial resolution, increases in P2 and N2 latencies were observed in the Pb group.

These data provide evidence that low-level neonatal Pb exposure produces toxic amblyopia. They also extend the findings that the scotopic system is more vulnerable to the toxic actions of Pb than the mesopic/photopic system and provide further support for the idea that all levels of the visual system are affected by developmental Pb exposure. Further experiments to determine the specific site(s) and mechanism(s) of action are in progress. Supported by grants ES 02713 and OH 07085.

23.3 CENTRAL EFFECTS OF LOW LEVEL DEVELOPMENTAL LEAD EXPOSURE ON OPTIC NERVE CONDUCTION AND THE RECOVERABILITY OF GENICULOCORTICAL RESPONSES IN HOODED RATS. D. Impelman*, C.L. Lear*, R. Wilson*, and D.A. Fox (Spon. B. Brooks). Div. of Tox. Univ. Texas Med. Schl,. Houston, TX 77025.

Long-term effects on visual functions in rats, following low-level neonatal lead (Pb) exposure suggest that clinical reports of irreversible impairments in spatial and temporal resolution properties of the visual system in children are partly due to changes in bioelectric properties of the optic nerve (ON) and in recoverability of visual cortical responses. Following neonatal Pb exposure latencies of the retinogeniculate and retinocollicular responses of flash visual evoked cortical potential (VECP) in adult rats are increased (Fox et al., Tox. Appl. Pharm. 40:449, 1977 and Neurobehav. Tox. 1:101, 1979). Latency increases in these pathways may reflect a decrease in the conduction velocity and excitability of the ON fibers since postnatal exposure to Pb produces hypomyelination and a decrease in fiber diameters of the ON (Tennekoon et al., Ann. Neurol. 5:558, 1979). Conduction velocities and chronaxy values obtained for the three ON conduction groups and the postsynaptic response latencies were measured in electrically evoked potential recordings from the LGN, superior colliculus (SC), and the striate cortex (CX) in adult control and Pb treated Long-Evans hooded rats. Female rats were exposed to Pb (or no Pb) from parturition to weaning (days 0-21) via the milk of dams consuming 0.2% Pb acetate solutions (or water). Electrical evoked recovery responses to double pulse stimulation of the ON were recorded because the similarity of electrical and flash evoked recovery functions suggested that Pb effects might be centrally mediated. Conduction velocities of the high (H) and middle (M) conduction groups were decreased in the Pb group with ranges of 9-15 m/sec vs 13-15 m/sec (Pb vs C) for the H group and 4.2-5.6 m/sec vs 5.2-7.0 m/sec for the M group. Chronaxy values for the same conduction groups were higher in the Pb group with ranges of 68-130 μs vs 39-59 μs for the H group and 125-148 μs vs 53-69 μs for the M group. Postsynaptic response latencies for the H and M conduction groups recorded form the LGN, SC, and CX were also increased but increases in the recovery response latencies and their amplitudes only occurred in the cortical recovery functions. Latency increases in the response components of the VECP recorded in Pb-exposed rats appear to be the result of a decrease in the conduction velocity and excitability of the fast and medium conduction groups in the ON. Increases in latency and amplitude of the recovery responses of the retinogeniculate component do not occur in the LGN which suggests that neonatal Pb exposure affects the cortical recovery mechanism. Supported by grants ES 02713 and OH 07085.

23.4 BEHAVIORAL DEFICITS IN MONKEYS EXPOSED TO LOW LEVELS OF LEAD SINCE BIRTH. Deborah C. Rice *and Steven G. Gilbert* (Spon: B. Weiss). Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2.

Monkeys (Macaca fascicularis) were dosed orally from birth with 0, 50, or 100 ug/kg/day of lead as lead acetate. Blood lead levels peaked at 16 or 22 ug/dl by 100 days of age for the two treated groups respectively, and decreased after weaning at 200 days of age to 12 ug/dl in both groups. Blood lead levels for control monkeys were always below the level of detection of the analytical procedure (5 ug/dl). When the monkeys were approximately three years old, they were tested on a two-choice discrimination and series of reversals paradigm. The monkeys faced a panel on which were a tube for delivery of fruit juice and two clear plastic response disks that could be backlit with various stimuli. The stimulus display consisted of a two-dimensional form (square or triangle) and color (red or green) on each disk. During the first part of the experiment, both disks were always backlit with red, and the monkey was required to respond on the disk displaying the square in order to receive a small amount of apple juice. The disk on which the correct stimulus appeared was varied randomly from trial to trial. When the monkey had learned the task (at least 9 out of 10 correct in a 10-trial block), the correct stimulus became the previously incorrect one, and the monkey had to relearn the task. A total of 20 such reversals were performed. For the two following tasks, the form and colors were displayed independently of each other, with one disk displaying one color and form, and the other disk the other possible pair. For the second task the monkey was required to attend to the colors on the disk and ignore the forms (initially red correct), while for the third task the forms were the relevant stimuli and the colors irrelevant (initially triangle correct). Twenty reversals were performed on each task. For each task, there were monkeys in the two treated groups whose performances were outside of control range on the reversal series, taking longer to learn the reversals and making more total errors than controls.

23.5 EFFECTS OF CHRONIC LOW LEVEL LEAD EXPOSURE ON THE PHYSIOLOGY OF INDIVIDUALLY IDENTIFIABLE NEURONS. Gerald Audesirk and Teresa Audesirk. Div. Biol. Sci., U. Missouri, Columbia, MO 65211.

Although chronic exposure to low levels of lead has been shown to be associated with behavioral deficits and neurochemical changes within the mammalian brain, relatively little is known of chronic lead's electrophysiological effects. Lead influences EEG measurements (Benignus, et al., 1981), suppresses evoked visual responses (Fox, et al., 1977), and inhibits spontaneous spiking in Purkinje cells of cerebellar grafts maintained in oculo (Palmer, et al., 1981). The present study utilized the nervous system of the pond snail Lymnaea stagnalis, with its individually identifiable neurons, as a model system in which to study lead effects on neuronal electrophysiology.

Juvenile Lymnaea were exposed to 1000 ug/l lead acetate in their aquarium water for 6 to 10 weeks; controls were identically reared but without lead. Isolated brains were dissected and pinned out in lead-free Ringer's. Intracellular recordings were made from the identifiable neurons RPeD1, VD1, and LP1; the pair VV1/VV2; and several (usually 3) neurons of the B and F clusters in each brain. Measurements were made of resting potential; action potential, overshoot, and undershoot amplitudes; action potential duration; rate of recovery of the undershoot; input resistance; time constant; spontaneous activity; and spike frequencies elicited by several intensities of intracellular current injection.

Multivariate analysis of variance of all parameters and all neuron types indicates that there is a significant overall effect of lead exposure ($p=0.0001$). Lead decreases spontaneous activity; reduces excitability to injected current (spontaneous activity subtracted out); decreases the input resistance; slows the rate of return of the spike undershoot to resting level; and increases the resting potential. Within these overall effects there is also a significant interaction between lead and neuron type ($p=0.02$). All neuron types in lead-exposed snails show, to some extent, a slowed rate of recovery of the undershoot and reduced spontaneous activity. However, B, F, and LP1 neurons are markedly less excitable when lead exposed, while the RPeD1 and VV1 neurons show no excitability change. The VV1 neurons also show no change in resting potential or input resistance.

These studies indicate that (1) characteristic changes occur in the electrophysiology of individual neurons as a result of in vivo lead exposure, and (2) different types of neurons differ in their response to chronic lead exposure. Further studies of affected and relatively unaffected neurons may lead to a more definitive understanding of the mechanism of action of chronic lead on the nervous system. (Supported by NIEHS grant 1 R23 ES02641-01.)

23.6 BIOCHEMICAL EFFECTS OF TRIETHYL TIN IN DEVELOPING RATS. R. Rocco and V. Sapirstein (spon: J. Blumberg) Dept. of Pharm. and Toxic Northeastern Univ., Boston, MA and Dept. of Biochem. E.K. Shriver Center, Waltham, MA.

Administration of triethyl tin (TET) to young animals induces a general retardation in growth, brain weight and a hypomyelination. The relative specificity of this toxin for oligo membrane formation suggests that the metabolic specialization of these cells, namely lipid synthesis, may contribute to their susceptability. These cells are enriched glucose 6P dehydrogenase (G6PDH) a function of which is to provide NADPH for fatty acid synthesis; glycerol P dehydrogenase, (GPDH).which provides glycerol phosphate for phospholipids; mouse mutant studies suggest that NADPH dependent fatty acid chain elongation is also enriched in these cells. Other enzymes highly enriched in oligos are CA and CNPase. We studed these and related enzyems in 21 day old rats that had received 3 consecutive IP injections of either distilled water or TET sulphate (2 mg/kg) on days 5,6 and 7. The TET treated animals had body and brain weights 85.5% and 49.6% of controls. The total protein /gm wet weight was 116% of control. The first two enzymes of the pentose shunt, G6PDH and 6P gluconate DH were unchanged in the TET animals. However, transketolase (TK) a rate limiting step in the non-oxidative portion of the shunt was reduced to 63% of control values. Because TK is a major source of glyceraldehyde 3 P which provides the substrate for GPD this enzyme was also measured. The activity in control animals was 61.0 + 3.2 nmoles/min/mg prot. while in the TET treated animals it was 45.9 + 3.2. The effects on TK and GPD imply a decreased capacity for glycerol-3-phosphate synthesis but no effect on the NADPH producing portion of the pentose shunt. We also measured levels of NADPH dependent isocitrate DH+NADPH-linked C18 fatty acid elongating activity but found no change from control values. CNPase was increased to 119% of control values but CA was significantly decreased in the TET animals (68% of control). We have found that inhibition of CA activity in brain slices by methazolamide decreases the labelling of lipids by 30% by $^{14}C\ HCO_3$. Decreases in this enzyme may compromise the ability of oligos to fix HCO_3 for the synthesis of lipids. None of the enzymes whose activities were found to be reduced are directly inhibited by TET in vitro.

These results indicate that TET causes a selective inhibition in activity of certain oligo enzymes required for lipid synthesis. The marked reduction in TK and GPD suggest that TET may exert an effect which results in the reduction of the non-oxidative limb of the pentose shunt, especially at the level of G3P incorporation into phospholipids. Supported by NS16186 and HD05515

23.7 TRIMETHYLTIN REDUCES BASKET CELL INHIBITION IN THE DENTATE GYRUS. R. S. Dyer, W. F. Wonderlin, T. J. Walsh*, and W. K. Boyes*†. Neurophysiology Branch, Neurotoxicology Division, Health Effects Research Laboratory, U. S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711.

Systemic administration of trimethyltin chloride (TMT) produces a pattern of neuronal loss in the limbic system similar to that produced by kainic acid. The time course of TMT-induced changes is more prolonged than kainic acid, and the seizures which follow injection do not occur for at least 3 days in rats. Because of the similarity in pathology, the mechanisms of action of these two substances may be similar. One proposed mechanism of kainic-acid-induced damage to pyramidal neurons in the hippocampal CA3 field involves increased activity in the mossy fiber-CA3 pyramidal cell pathway. Sloviter and Damiano (Neuropharmacol. 20: 1003, 1981) have shown that kainic acid reduces basket cell recurrent inhibition of the dentate granule cells, thereby increasing mossy fiber activity despite constant perforant path (PP) input. We investigated the influence of TMT upon this pathway. Adult male Long-Evans hooded rats were implanted with bipolar stimulation electrodes in the PP and a monopolar recording electrode in the hilus of the dentate gyrus (referred to frontal sinus). Following a week of postsurgical recovery, baseline recordings were obtained in awake unrestrained rats. A mean of 32 responses was obtained following stimulation of the PP electrodes at an intensity that was supramaximal for appearance of the dentate granule cell population spike. To assess the amount of recurrent inhibition, stimuli were presented in pairs, with a 20 msec interstimulus interval within the pair, and a 2 sec interval between pairs. In control rats, recurrent inhibition greatly reduced the amplitude of the population spike produced by the second of the pair of stimuli. In TMT-treated rats, however, recurrent inhibition was attenuated within 2 hrs after an i.p. injection of 6 mg/kg and almost completely abolished within 3 days. PP stimulation occasionally produced multiple granule cell population spikes in TMT-treated rats. Thus, the mechanism of TMT neurotoxicity may parallel that of kainic acid: reduction of basket cell recurrent inhibition, resulting in an increase in mossy fiber activity and consequent damage due to overstimulation of CA3 pyramidal cells. The rapid onset of these changes suggests a pharmacological effect of TMT, perhaps by blocking the GABA receptors of the recurrent inhibitory pathway (Matthews et al., Neuropharmacol. 20: 561, 1981).

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23.8 NEURAL AND BEHAVIORAL TOXICITY OF TRIMETHYLTIN IN DEVELOPING RATS. Patricia H. Ruppert, Karen F. Dean* and Lawrence W. Reiter. Neurotoxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Acute exposure to triethyltin (TET) on postnatal day 5 produces preweaning deficits in rope descent, a decrease in both brain and body weight, and an increase in motor activity which persists through adulthood (Reiter et al., Neurobehav. Toxicol. Teratol. 3: 285-293, 1981). In subsequent studies, we found reduced milk bands in TET-dosed pups, and a reduction in the adult acoustic startle response. To extend our investigations on the developmental toxicity of trialkyltins, we examined the effects of trimethyltin (TMT) on these variables. On day 5, rat pups (LE) received a single intraperitoneal injection of either 0 (saline), 4, 5, or 6 mg/kg TMT-hydroxide as the base. A within-litter design was used for dosing: one male and one female from each litter received each dose and, therefore, each litter contained all treatments. The size of the milk bands (a measure of milk consumption) was significantly reduced in 6 mg/kg TMT pups 48-96 hr after dosing, while in 5 mg/kg TMT pups, milk bands were reduced 96 hr after dosing only. During the preweaning period, dosages of 5 and 6 mg/kg TMT also produced growth retardation (with recovery by day 30) and impaired performance in rope descent. As adults, motor activity in a figure-eight maze was increased for 6 mg/kg TMT animals. The startle response to an acoustic stimulus (a 13kHz, 120dB(A) pure tone) was also affected by TMT treatment when measured both during ontogeny and in adulthood. During development, on days 10-21, all dosages of TMT reduced the number of responses during a 30 trial session in both males and females. The amplitude of positive responses was significantly reduced for the 6 mg/kg dose on days 12, 18, and 20. As adults, the amplitude of startle was decreased at all dosages for males but not for females. These behavioral changes were accompanied by decreases in adult brain weight for both sexes. Whole brain weight was decreased following 4 (4%), 5 (7%), and 6 (16%) mg/kg TMT. The weight of the olfactory bulbs was also reduced by 4 (8%), 5 (9%), and 6 (13%) mg/kg TMT. Hippocampal weight was decreased following 5 (22%) and 6 (39%) mg/kg TMT, while decreased cerebellar weight (8% at 6 mg/kg TMT) was not significant due to variability. Although the neuropathology produced by TET and TMT in adults is dissimilar, these alterations in behavior, body weight, and brain weight parallel those produced by TET on postnatal day 5.

23.9 **RELATIVE REFRACTORY PERIOD OF NERVE AXONS AS A DISCRIMINATOR OF NEUROTOXICITY.** R.J. Anderson. Dept. Pharmacology, George Washington Univ., Washington, DC 20037.

Because the relative refractory period is a logarithmic function, it has been a difficult measurement to handle statistically. Most investigators have limited their assessment to (1) determining the absolute refractory period and/or (2) the duration of the entire relative refractory period. We present here a simple method for conversion of the relative refractory period to a linear representation, which is more easily handled statistically and which may have particular utility in assessing neurotoxic agents.

The rat sciatic nerve was stimulated with twin pulses at intervals from 0.8 to 10 msec to evoke a pair of compound action potentials, the second of which fell within the relative refractory period. The decrement in amplitude and area of this response was used to generate the best fitting refractory period line according to the equation:

$$Y = A \exp(BX)$$

where Y is the decremented action potential at each interval, X, and A and B are coefficients found by solving the equation for stimulus intervals yielding responses between 20 and 80% of the control response. A straight line is then fitted using the calculated coefficients (A and B) and Y values of 25, 35, 50, 65, and 75% and their corresponding interstimulus intervals. The slope and intercepts of this straight line therefore define the complete characteristics of the relative refractory period and can be compared between control and test groups. Since variations in this line are quite small among control animals (for a given strain, weight and age), deviations from the control line are easily recognized and interpreted.

Acrylamide, phenol, trichlorfon and erythrosin were used as test compounds because each produces a different type of neurotoxicity. Phenol, a demyelinating agent, greatly prolonged the refractory period, the slope of which correlated with the degree of histopathology. Trichlorfon, an organophosphate which induces delayed neuropathy, showed a progressive shift in the refractory period during the development of the neuropathy. Acrylamide, an agent whose first neurotoxic effects are at the nerve terminal, showed no change in refractory period until the axon itself was structurally damaged. Erythrosin, an agent which seems to increase neuroexcitability, showed changes in relative refractory period which suggest an enhancement of axonal excitability particularly to high frequency neurotransmission.

These results suggest that the linear representation of the relative refractory period can serve as a sensitive, discriminative and quantitative measure for detecting neurotoxicity. (Supported by NIEHS.)

23.10 ORAL DOSING OF RATS WITH POLYCHLORINATED BIPHENYLS INCREASES URINARY HOMOVANILLIC ACID LEVELS. Richard F. Seegal, K.O. Brosch* and Brian Bush*. Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201.

Polychlorinated biphenyls (PCBs) are widespread environmental contaminants demonstrated to produce adverse health effects in both man and animal (Fishbein, 1974; Hutzinger et al., 1974). Nervous system involvement includes disturbances in motor performance (Tilson et al., 1979), increased frequency of convulsions and neuropathological change in mouse spinal cord and limbic system structures (Chou et al., 1979) and alterations in whole brain catecholamine concentrations (Heinz et al., 1980).

4-hydroxy-3-methoxyphenylacetic acid (HVA), one of the major end products of dopamine (DA) metabolism in the rodent brain, can be used to estimate dopamine turnover (Westerink, 1979). When measured in the periphery, it co-varies with central changes following pharmacological, and electrophysiological manipulation (Sternberg et al., 1981).

Adult male Sprague-Dawley rats weighing between 250-300 g were singly housed in metal rodent metabolism cages. Animals received either a single oral dose of a mixture of Aroclor 1254 and 1260 (total dose = 500 or 1000 mg/kg) or corn oil vehicle and were followed for 21 days post-gavage. Food and water consumption, body weight and urinary HVA concentrations were measured every 3 days. HVA levels were determined by HPLC with electrochemical detection.

No significant differences in body weight, food and water consumption, urine volume or 24 h urinary HVA production were noted between any of the groups prior to gavage. Following treatment, there were transient reductions in body weight, and food and water consumption in the high PCB-exposed group. No significant changes in any of these measures were noted in the low-PCB or corn oil exposed groups. 24-h urinary HVA production was increased by approximately 175% in the low-PCB group, but returned to control levels after 6 d. 24 h HVA production increased by 215% in the high-PCB exposed group and remained elevated throughout the experiment. No significant differences in 24 h urine production were noted following treatment.

These results demonstrate a dose-dependent long-term change in dopaminergic function following a single oral exposure to a complex mixture of PCB congeners and are consistent with results obtained by Rosin and Martin (1981). These authors found a dose-dependent stimulation of release and inhibition of uptake of DA and other neurotransmitters in PCB-treated mouse brain synaptosomal preparations. Thus, peripherally obtained measures of DA-ergic function may serve as a practical, sensitive and objective index of exposure to PCBs and other putative neurotoxins.

23.11 LONG TERM STABILITY OF FUNCTIONAL DEFICIT IN LISSENCEPHALIC FERRETS PRODUCED BY A SINGLE PRENATAL INJECTION OF METHYLAOZXY-METHANOL ACETATE (MAM Ac). R. Haddad, R. M. Dumas* and A. Rabe. Neuroteratology Laboratory, NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

The ferret has a gyrencephalic brain, but the normal convolutional development can be prevented by giving the pregnant jill a single injection of MAM Ac (15mg/kg, IP) on gestation day (GD) 32 (mating = GD 1). (Full term is 42 days.) All the progeny are then lissencephalic, i.e., smooth brained, and hydrocephalic.

Lissencephalic ferres, tested at about one year of age (sexual maturation is 7-8 months) have been shown to be severely impaired in their performance on several maze learning tasks (Haddad et al., 1979, Neurotoxicology, 1, 171). Neuropathologic examination of the brains of these animals showed a severe hydrocephaly which led us to question whether the functional deficits found reflected degenerative brain changes rather than, or as much as, a developmental deficit. For this reason, a new group of treated and normal ferrets were evaluated at weaning (6-7 weeks of age). The lissencephalic weanlings displayed deficits comparable to those found in the adult (Haddad et al., 1980, P. 171 in FDA Symposium on the Effects of Foods and Drugs on the Development and Function of the Nervous System, HHS Publication No. (FDA) 80-1076).

Most of the latter group of ferrets were subsequently implanted with screq electrodes for chronic EEG recordings. They were operated between one and two years of age. EEG abnormalities were found in the lissencephalic ferrets (Lee et al., 1981, Teratology, 24, 13A). They were also found to differ in their seizure threshold and to be less responsive to anticonvulsant drugs (Majkowski et al., in preparation).

After completion of these tests, at about three years of age, all of the implanted ferrets were given another maze learning test. Additional unimplanted lissencephalic and normal ferrets of comparable age that had had neither previous testing nor any drug treatments were also tested. The performance of the three year old lissencephalic and normal ferrets was comparable to that of those previously tested at weaning and at one year of age. The lissencephalic ferrets again differed sharply from the normal animals ($p < .001$), making many more errors, while the range of errors for both the treated and normal groups was similar to that of the animals tested at younger ages.

The lissencephalic ferret thus provides an animal model for one form of mental retardation that should be useful for pharmacologic investigations since the functional deficit appears to be quite stable over a period of years.

23.12 A HISTOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERIZATION OF GENTAMICIN INDUCED HINDLIMB PARALYSIS IN THE RAT. J.M. Tolliver* and J.E. Warnick. Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201.

The intracisternal administration of gentamicin (GEN) to a patient and to rabbits induces similar histological lesions with tetraplegia occuring in rabbits (Ann. Neurol. 4:564, 1978). To further define the neurotoxic actions of GEN, the effect of subdural administration of GEN sulfate (GEN-S) on the rat spinal cord and extensor digitorum longus (EDL) muscle was investigated. A laminectomy was performed at L_1 under ether anesthesia and a fine glass capillary micropipette was inserted through the dura. Either GEN-S (3 μ moles in 20 μl) or physiological saline (20 μl) was injected under the dorsal roots. The incision was closed and animals recovered from anesthesia in about 30 min, upon which a flaccid hindlimb paralysis lasting about 3 h was observed. Muscle tone then gradually returned and within 15 h mobility was normal. Between 24 and 36 h after injection, a period of hyperexcitability characterized by hindlimb tremors and lower spinal seizures was evident. This was followed within 24 h by flaccid hindlimb paralysis that appeared to be permanent. In contrast, control animals receiving physiological saline showed no signs of motor abnormalities at any time after injection. Within 24 h of injection, a proliferation of small phagocytic cells was noted in the lumbar spinal cord. By 48 h axonal swelling, breakage and end bud formation had started to appear. From 3 to 7 days after injection, extensive chromatolysis of motoneurons was observed. With the exception of an occassional inflammatory response, spinal cords from control rats were histologically normal. For up to two days after injection, resting membrane potentials (RMP) in EDL muscles of sham-operated control and treated rats were similiar. At 4 and 7 days, RMPs in EDL muscles of GEN-S treated rats had decreased from -78 mV to -62 mV and -58 mV, respectively. In contrast, the RMP for EDL muscles of sham-operated controls was -77 mV. At 7 days after injection miniature endplate potentials could not be detected and tetrodotoxin resistant action potentials and extrajunctional acetylcholine sensitivity were present in EDL muscles of treated rats. These signs of functional denervation were not found in control EDL muscles up to 7 days after injection. GEN-S thus produces a profound spinal cord neuropathy with subsequent skeletal muscle denervation. The initial paralysis may result from GEN-S induced blockade of transmitter release in the cord whereas the final paralysis may reflect a neuronal-glia interaction. (Supported in part by U.S. Army Contract DAMD-17-81-C-1279.)

23.13 ACUTE DOSES OF NICOTINE INDUCE DEGENERATION IN REUNIENS NUCLEUS OF THALAMUS, R. C. Switzer IV* and R. C. Switzer III. Knoxville, TN, and Comparative Animal Research Laboratory, Oak Ridge Associated Universities, Oak Ridge, TN 37830.

Nicotine is a well-known stimulant of the CNS, while another nicotinic acid analog, 3-acetyl pyridine, has been shown to destroy specific neuronal populations such as inferior olive and lateral geniculate. We report here the specific destruction of neurons in reuniens nucleus of thalamus in rat following a single dose of nicotine. Each of six male Sprague-Dawley rats (200-250 g) was given a single intraperitoneal injection of a nicotine solution (2 mg/ml) at one of the following doses: 0.5, 1.0, or 2.0 mg/kg. After three days the rats were perfused under heavy pentabarbitol anesthesia, and their brains sectioned and processed according to the cupric-silver procedure of de Olmos.

Degeneration as revealed by argyrophilic cell bodies and surrounding dendritic fragments was found throughout the entire rostro-caudal extent of reuniens; however, not all cells were affected. At the coronal level where reuniens is widest, degenerated cell bodies were present as bilaterally symmetric groups, suggesting that a specific subpopulation of reuniens was affected. Degenerating axons could be traced from reuniens following the efferent pathways described by Herkenham (J. Comp. Neurol., 177:589-610, 1978). The rat receiving 0.5 mg/kg of nicotine displayed no degeneration.

Although it remains to be established if lower doses given chronically would yield the same destruction, these results reveal the reuniens nucleus as particularly susceptible to nicotine. Lower doses (less than 1 mg/kg) may not be lethal to neurons of reuniens but the physiology of these neurons may be perturbed to a greater extent than neurons in other CNS areas. Such an effect is of particular interest because of the role reuniens appears to play in moderating ascending neural activity from organs of the viscera, such as heart and lung, en route to limbic areas. The importance of thalamic influence on the viscera has been indicated by the findings that bilateral destruction in thalamus causes myocardial necrosis (McGeer and McGeer, CRC Crit. Rev. Toxicol., 10:1-47, 1982).

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23.14 NEUROTOXICITY OF TUBULIN BINDING AGENTS IN RAT HIPPOCAMPUS. Richard B. Goldschmidt*, John H. Gilmore*, S. Scott Lasher* and Oswald Steward. (SPON: Christine Phillips). Depts of Neurosurgery & Anatomy, University of Virginia School of Medicine, Charlottesville, VA 22908

Injection of colchicine into the dentate gyrus of adult rats preferentially destroys dentate granule cells (Goldschmidt & Steward, PNAS 77: 3047, 1980). In an effort to elucidate the mechanism of action of colchicine, we have tested several tubulin binding agents and the non-binding analogs of colchicine - β and γ lumicolchicine, and observed whether they also induce preferential destruction of granule cells.

The drugs to be tested were prepared in equimolar concentrations to the usual dose of colchicine (about 9.5 nmoles/injection). Colchicine, colcemid, vinblastine and vincristine were dissolved in deionized water, while podophyllotoxin and β and γ lumicolchicine were dissolved in .95ml water and .05ml 75% ethanol. Adult male rats were given a single injection (.7µl) into the dorsal hippocampus and allowed to survive between 2 and 30 days.

Vincristine proved to be extremely toxic even at half the initial concentration. It killed neurons of all kinds for several mm from the injection site. Vinblastine was relatively selective in damaging granule cells, but was not as potent as colchicine in that the effect spread less than a mm from the injection. Colcemid and podophyllotoxin caused very little if any damage. Lumicolchicine injections and control injections of the vehicle containing ethanol caused no cell damage beyond the mechanical effects of inserting the syringe.

This ordering of the effects of these related drugs appears to correlate with the persistence of their binding to tubulin (Dustin, Microtubules, 1978). This suggests that granule cell death may be related to the length of time tubulin molecules are bound. However, it is also possible that differences in permeability to these different drugs might account for our results.

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23.15 ON THE MECHANISMS OF GLUTAMATE NEUROTOXICITY. N. Brookes, R.C. Wierwille* and A. Boyne. Dept. of Pharm. & Exp. Ther., Univ. of MD Sch. of Med., Baltimore, MD 21201.

It is possible that glutamate is an endogenous neurotoxin in degenerative diseases of the CNS or that it mediates effects of exogenous neurotoxins. However, the way in which glutamate kills neurons is unclear. Monosodium glutamate (MSG) at 0.2 mM or more selectively destroys spinal cord (SC) neurons in primary cell cultures derived from the spinal cords of fetal mice (Brookes and Burt, Dev. Neurosci. 3:118, 1980). Neuronal death was quantitated by the reduction in uptake of [^{3}H]2-deoxyglucose (2-DG) by the cultures (Brookes and Burt, Soc. for Neurosci. Abstr. 6:180, 1980). MSG-susceptible neurons accounted for 50-75% of TEA-stimulated 2-DG uptake in suitable control cultures. This component of 2-DG uptake ('neuronal 2-DG uptake') was eliminated by addition of MSG 1 mM either for 10 min immediately before 2-DG uptake measurement or during the 20 min uptake period itself. On the other hand, a 1 min exposure to MSG 1 mM did not affect neuronal 2-DG uptake whether measured immediately or 2 days later. MSG did not accelerate the short-term leakage of 2-DG from pre-loaded SC cultures, yet no conditions were found in which an initial phase of MSG-induced neuronal excitation was demonstrable as increased 2-DG uptake.

The neurotoxic action of MSG 1 mM persisted in the presence of TTX 0.1 µM and in a calcium-free salt solution containing EGTA 1 mM. However, substitution of choline chloride for sodium chloride protected SC neurons against MSG. This appears to be an effect of choline rather than low sodium concentration since neither substitution with sucrose nor HEPES was protective. To determine whether short-term depolarization alone is sufficient for neurotoxicity, SC cultures were exposed to a solution containing 100 mM potassium. Initial studies reveal comparable neurotoxic effects of MSG 1 mM and 100 mM potassium as judged by 2-DG uptake, light and electron microscopy. The extent of comparability requires further examination.

Ultrastructural correlates were obtained by quick-freezing SC cultures on glass discs followed by freeze-substitution in tetrahydrofuran. Glial cells and dorsal root ganglion neurons appeared unaffected whereas other neuronal cells developed swollen endoplasmic reticulae and were extensively vacuolated 3 hr after a 20 min exposure to MSG 1 mM. During the first 10 min, mitochondria of affected cells became loaded with apparent calcium deposits which grew progressively larger. A similar loading occurred in neurons when the cultures were exposed to solutions containing 100 mM potassium. (Supported by USPHS grant MH29011 and U.S. Army contract DAMD 17-81-C-1279.)

23.16 NEUROCHEMICAL AND TOXICOLOGIC EFFECTS OF ANTICONVULSANT DRUGS ON CEREBRAL CORTICAL CULTURES. Elaine A. Neale, Phyllis K. Sher* and Phillip G. Nelson. Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20205

Anticonvulsants are commonly used for the treatment of major and minor motor seizures in young children, yet there is little consensus as to their mechanism of action or their toxicity to the developing nervous system. In an attempt to evaluate these issues, we have exposed dissociated cell cultures obtained from fetal mouse cerebral cortex to six drugs at approximately twice the high serum therapeutic concentration between days 9 and 20 in culture: phenytoin (PY) 40 µg/ml; phenobarbital (PB) 80 µg/ml;carbamazepine (C) 24 µg/ml; valproic acid (V) 200 µg/ml; diazepam (D) 5 µg/ml; and ethosuximide (E) 200 µg/ml. Following drug exposure, cultures were assayed in situ for ^{125}I-tetanus toxin labeling (TET), high affinity uptakes of ^{3}H-GABA and ^{3}H-β-alanine, choline acetyltransferase (CAT) activity, ^{3}H-benzodiazepine (BDZ) binding, clonazepam displaceable (CLO) BDZ binding, total protein and neuronal cell counts. Results for drugs used primarily to treat major motor seizures (PY, PB, C) indicated that PY-treated cultures evidence greater neuronal toxicity than PB or C (cell counts: 53 ± 3.7, 63 ± 3.9, 88 ± 4.7; CLO: 64 ± 2.9, 86 ± 4.9, 92 ± 7.7 percent of control ± SEM, respectively). For those drugs used to treat minor motor seizures (V, D and E), V and D showed greater neuronal toxicity than E (cell counts: 74 ± 2.9, 79 ± 3.2, 94 ± 6.3 percent of control ± SEM, respectively). In the latter group of drugs, CLO was non-detectable for D-treated cultures, 47 ± 3.9 for V-treated cultures and 79 ± 4.4 percent of control for E-treated cultures. This disproportionate reduction in both specific BDZ and CLO binding following V, D, or E exposure may relate to the mechanism of action of this group of drugs as such a reduction was not present in PY, PB, or C-treated cultures. Similarly, β-alanine uptake, largely a marker of GABA uptake into non-neuronal cells, was significantly depressed relative to neuronal GABA uptake in V-treated cultures and slightly depressed in D-treated cultures, but was not depressed in PY, PB, C, or E-treated cultures. CAT activity, and to a lesser extent, TET and CLO (at least for PY, PB and C) most accurately reflected variations in neuronal cell counts. Each of these parameters was thus depressed in proportion to the presumed relative toxicity of each drug. Results with this experimental system demonstrate a different profile of effects for the two groups of anticonvulsants, and suggest a hierarchy of toxicity within each group.

23.17 NEONATAL CHLORDECONE EXPOSURE ALTERS LEARNING/RETENTION OF ACTIVE AVOIDANCE IN THE RAT. C.F. Mactutus and H.A. Tilson. Lab. Behav. Neurol. Toxicol., NIEHS, Research Triangle Park, NC 27709.

Neonatal exposure of rats to chlordecone, during a period of neuroendocrine differentiation, alters cognitive and affective measures of passive avoidance behavior, particularly in male rats (Neurosci. Abst., 7:892, 1981). To the extent that a true associative deficit underlies the previous observation, interference with acquisition and retention of active avoidance should also be observed.

Fischer-344 rat pups were administered a single s.c. injection (20 µl) of either dimethylsulfoxide (DMSO) or 1 mg/pup of chlordecone dissolved in DMSO on postnatal day 4. Body weights were slightly, but significantly, depressed for chlordecone-exposed males on day 14 (9.9%) and for chlordecone-exposed males and females on days 21 (9.3% and 6.5%) and 35 (6.8% and 6.6%). Half of the pups were trained (day 18) on one-way avoidance (OWA). Chlordecone treatment increased the number of trials needed to attain the acquisition criterion; an effect most pronounced in the female pups. A retention test after a 72-hr interval indicated a trend for more rapid responding by chlordecone-exposed females relative to controls, with no difference between the male groups. Acquisition of two-way avoidance (TWA, days 28-30) found little influence of chlordecone treatment among pups which had no prior OWA experience. However, prior OWA training increased avoidance responding of chlordecone-exposed males and decreased avoidance responding of chlordecone-treated females, relative to vehicle-injected siblings. Perhaps most importantly, vehicle control pups demonstrated a directional bias to make an avoidance response from a small to a large compartment, while chlordecone-treated pups executed their avoidance responses in both directions at the same rate. These latter two observations also characterized TWA performance when a "reversal" procedure was used. A final extinction test indicated that chlordecone-treated pups made fewer responses both during and between trials, with the latter difference significantly greater between the female treatment groups than between the male groups. Plasma steroid levels of male and female chlordecone-treated pups were 15-16% higher than vehicle control values when the animals were sacrificed 5 min after the extinction test.

In summary, the unidirectional response bias in the TWA procedure by the vehicle control pups which had prior OWA training indicated retention of previous training which was not observed in the chlordecone-treated pups. The greater plasma steroid levels of chlordecone-treated pups over vehicle controls is consistent with the hyper-responsiveness previously noted following such exposure and indicates that increased "emotionality" also played a role in the expression of the observed behavior.

23.18 ALTERED BRAIN GROWTH IN BENZENE EXPOSED RAT PUPS, F. Petracca* and J. Diaz. (SPON: S. R. Burchfield) Dept. of Psychology, University of Washington, Seattle, Washington, 98195.

Benzene is an aromatic hydrocarbon frequently encountered in the workplace and home. Toxic effects of this chemical have been well documented in adults, and the National Institute of Occupational Safety and Health has established standards for regulation of workers' exposure to this substance. However, controversy remains regarding the effects of benzene on the developing organism (Mehlman, M., et. al., J. Env. Pathol. Toxicol., 4:123, 1980.; Tilson, H., et. al., Neurobehav. Toxicol., 2:101, 1980.). The purpose of this study was to determine possible effects of benzene when administered during the stage of most rapid brain growth in the rat pup. This stage, called the brain growth spurt (Dobbing, J. & Sands, J., Arch. Dis. Childhood, 48:757, 1973.), occurs pre- and postnatally in humans but entirely postnatally in rats.

4-day-old male Long-Evans rat pups were weighed and culled to litters of 8 and assigned to one of the following groups: 1) pups receiving 550 mg/kg of benzene, on days 7 through 11; 2) pups receiving 225 mg/kg of benzene, on days 7 through 11; 3) pups receiving corn oil vehicle, on days 7 through 11.

From days 4 through 18, pups were weighed daily and were administered a battery of reflex tests, including righting, negative geotaxis, cliff avoidance and free-fall righting. The appearance of teeth and eye opening were noted. On each of days 7 through 11, pups received a subcutaneous injection of either dose of benzene or corn oil vehicle. On day 17, all animals were tested in an open field apparatus. On day 18, each animal was sacrificed. The brain, liver, kidney, and spleen were removed and weighed.

There was a significant decrease in whole brain weights and cerebellar weights for the two benzene treated groups compared to controls ($p<.005$). No significant differences were seen in day 18 body weights, nor in liver, kidney, and spleen weights.

Behaviorally, the benzene treated groups showed indicators of intoxication within 30 minutes after injection. However, no significant differences were seen in ontogeny of reflexes, nor were they seen in day 17 open field measures.

The results of the present study suggest that benzene may have specific toxic effects on neural development. Peripheral organ weights were not affected by either dose of benzene, but whole brain and cerebellar weights were decreased in the two benzene exposed groups. Furthermore, these data suggest that the long term behavioral effects of early benzene exposure documented by Tilson et. al. (Neurobehav. Toxicol. 2:101, 1980) may be mediated by disruptions in CNS development.

23.19 DESTRUCTION OF GABA NEURONS IN THE AMYGDALA AND PYRIFORM CORTEX BY FOLIC ACID INJECTIONS INTO THE SUBSTANTIA INNOMINATA. P. L. McGeer, E. G. McGeer, T. Nagai* and P. T.-H. Wong. Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B. C., V6T 1W5.

Unilateral lesions were induced in the substantia innominata (SI) of rats by three methods: electrocoagulation; injection of kainic acid (KA) (2 nmol); injection of folic acid (FA) (50-200 nmol). Histological examination by cresyl violet and GABA-transaminase staining and biochemical evaluation, by glutamic acid decarboxylase (GAD) and choline acetyltransferase (ChAT measurements, and by [^{3}H]QNB and [^{3}H]benzodiazepine ([^{3}H]BZD) binding studies, were undertaken of the SI and several remote areas. Injections of FA into the SI produced much less local but more severe distant neuronal damage than did injections of KA, as previously reported by Olney et al.[1] Both produced sustained epileptiform activity. Electrolytic lesions, on the other hand, produced only local neuronal damage and no epileptiform activity. Biochemical measurements of GAD and histochemical staining for GABA transaminase indicated many of the neurons in the distant areas affected following FA injections were GABAergic, but cholinergic neurons were relatively spared. Damage to the cortical areas was heaviest in the superficial layers. The most severe distant damage after FA was noted in GAD in the amygdala and pyriform cortex, and to a lesser extent in the frontal, entorhinal and temporal cortices, and in the thalamus. The striatum and hippocampus were spared. The distant damage, except in the thalamus, seemed to parallel the density of cholinergic innervation from the SI as revealed by relative drops in ChAT following KA injections into the SI. Reduction in both seizures and remote damage was brought about by pretreatment of the animals with valium (20 mg/kg) or scopolamine (50 mg/kg). The protective action of scopolamine is further evidence that cholinergic neurons may mediate much of the remote damage to GABA neurons although they themselves are little affected. Falls in [^{3}H]QNB but not [^{3}H]BZD binding in the affected areas are consistent with such a mechanism. Distant effects of injections of FA into the amygdala or striatum were comparable in kind but much less in magnitude to those after SI injections.

[1] Olney, J.F., Fuller, T.A., de Gubareff, T. and Labruyere, J., Neurosci. Lett., 25:185-191, 1981.

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23.20 EFFECTS OF CHRONIC PRENATAL AMPHETAMINE EXPOSURE ON CENTRAL DOPAMINERGIC AND SEROTONERGIC SYSTEMS IN THE RAT. E. Snowhill-Gisclon* and J.W. Gibb. (SPON: S. Stensaas) Dept. of Biochemical Pharmacol. and Toxicol., Univ. of Utah, Salt Lake City, UT 84112.

Administration of amphetamine (AMPH) or methamphetamine (METH) to adult male rats has been shown to produce significant decreases in the activities of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) (Hotchkiss and Gibb, JPET, 214:257, 1980; Bakhit et al., Neuropharm., 20:1135, 1981). Enzyme activities are depressed as long as 110 days after chronic or subacute drug administration (Ellison et al., Science, 201:276, 1978; Hotchkiss et al., Life Sci., 25:1373, 1979).

This study was designed to monitor similar neurochemical changes that might be expected in the dopaminergic and serotonergic systems of offspring of female rats exposed chronically to AMPH during gestation.

Timed-pregnant rats were injected chronically with AMPH under the following regimen: 0, 0.5, 2.0, or 5.0 mg/kg, s.c. every 12 hours from day 7 of gestation until term. Dosing was stopped 12-18 hours before expected delivery time. Control and treated groups did not differ in length of gestation or in body weights of 3-week old offspring. Male offspring were sacrificed by decapitation between 2 and 8 weeks of age and brain areas (cerebral cortex, neostriatum, hypothalamus, and hippocampus) were dissected out for neurochemical analysis.

TPH activity was measured by a $^{14}CO_2$-trapping method (Hotchkiss et al., ibid.); TH activity was measured by the method of Nagatsu et al. (Anal. Biochem., 9:122, 1964). Neurotransmitters and metabolites were quantitated using high performance liquid chromatography with electrochemical detection (K.E. Moore, personal communication), measuring DOPA, dopamine, DOPAC, HVA, 5-HTP, 5-HT, and 5-HIAA simultaneously in a single brain homogenate sample.

At 5 weeks postpartum, dams showed depression of the dopaminergic system, with dopamine decreased to 73%, DOPAC to 75%, and HVA to 72% of respective controls. 5-HT and 5-HIAA in treated dams were not different from controls. Animals allowed to recover until 12 weeks postpartum showed both transmitter systems at normal levels. Two-week old offspring showed significant dose-dependent decreases in TPH to 69% and 54% of control for 2 mg/kg and 5 mg/kg doses, respectively. Corresponding TH activities were 85% and 80% of controls. By 8 weeks of age these activities had returned to normal, and neurotransmitter levels were not different from control.

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24.1 ALTERATIONS IN THE POWER SPECTRA OF KINDLED HIPPOCAMPAL SEIZURES IN ADULT RATS EXPOSED TO LEAD NEONATALLY. M. McCarren*, G. A. Young and C. U. Eccles. Dept. of Pharmacology and Toxicology, Univ. of Maryland School of Pharmacy, Baltimore, MD 21201.

The present study was designed to detect long-term effects on hippocampal function following a brief postnatal lead exposure and to compare the sensitivity of this test with others. Previous work in our laboratory (McCarren, M. and Eccles, C.U., Soc. Neurosci. Abst. 289:15, 1981) demonstrated that acute hippocampal afterdischarges are altered in lead-treated animals. It was of interest whether lead could affect kindling of hippocampal seizures. Power spectral analysis of the kindled seizure was utilized because of the sensitivity of this technique in other investigations of central nervous system function.

Sprague-Dawley rats were exposed postnatally to lead indirectly via the dam's milk. Dams' drinking water contained 1.0, 2.5, or 5.0 mg/ml of lead acetate or 1.25 mg/ml of sodium acetate. Pups were weaned to tap water at 22 days of age. At 3 months of age, adult males were prepared with chronic indwelling bipolar hippocampal and monopolar cortical electrodes. Animals were kindled by stimulating in the hippocampus at twice threshold (2 sec train of 50 Hz, 0.1 msec biphasic square wave pulses) at hourly intervals. Seven stimulations were applied per day, and this continued daily until the rat experienced his first stage 4 convulsion (forelimb clonus with rearing). From that time on, the animal received only one stimulation per day. Spectral analysis (Fast Fourier Transform) was performed only on stable, stage 5 (forelimb clonus with rearing and falling) kindled seizures; the data were normalized with respect to afterdischarge duration. Power in selected frequency bands was expressed as percent of total power during the primary afterdischarge.

No significant differences in kindling rates were detected among the groups, although there was a tendency for lead animals to kindle more slowly. Spectral analysis revealed that a greater percentage of total power resides in the lower frequency bands in lead animals (1-3 Hz, 3-5 Hz, 5-7 Hz) as compared to controls. The reverse is true at higher frequencies, where controls had higher percentages of power (13-15 Hz, 15-17 Hz, 17-19 Hz, 19-21 Hz, 21-23 Hz, 23-25 Hz). These data indicate that power spectral analysis is a sensitive technique for detecting subtle lead toxicity.

24.2 KINDLED MOTOR SEIZURES ARE ABOLISHED BY MICROINJECTION OF MUSCIMOL INTO VENTRAL MIDBRAIN TEGMENTUM. J. McNamara, M. Iadarola, L. Rigsbee*, and M. Galloway*. Epilepsy Center, Depts. of Med. and Pharm., Duke Univ. and VAMC, Durham, NC 27705.

The term kindling refers to a phenomenon whereby repeated periodic administration of an initially subconvulsive electrical stimulus results in progressively increasing seizure activity, culminating in generalized seizures. Elucidating the network of neural circuits responsible for kindling is essential to understanding the basic cellular and molecular mechanisms. Activation of brain stem structures parallels the appearance of motor seizure activity. Analysis of brain stem structures in 2-deoxyglucose autoradiographs of kindled seizures discloses increased metabolic activity in the substantia nigra (SN). Microinjection data implicate SN as a key site of GABA mediated anticonvulsant activity in other seizure models. Systemic administration of gamma vinyl GABA, an irreversible GABA transaminase inhibitor, blocks the motor component of kindled seizures. We therefore hypothesized that pharmacologic enhancement of GABA-ergic synapses in SN would abolish motor kindled seizures.

Male rats underwent stereotaxic implantation of electrodes in amygdala and guide cannulas in several brain structures. Electrode and cannula placements were histologically verified in all animals. Electrical stimulations were administered until motor seizures of constant duration were reliably elicited.

The effects of microinjection of saline and muscimol (M) (a GABA agonist) on motor seizure duration were determined. Animals were gently restrained during injection. M injections into SN induced stereotypies and turning behavior which were well developed by 30 min. after injection. Therefore drug effects were monitored by stimulation administered 30 min. after injection. Motor seizure duration was not altered by microinjection of saline into midbrain (x seconds $\pm$ SEM, baseline 30.5 $\pm$ 1.0, saline 31.7 $\pm$ 2). By contrast, microinjection of M (50 ng in 0.5 µl of saline) bilaterally into SN abolished motor seizures in every animal tested (baseline 27.6 $\pm$ 1, M 0 $\pm$ 0). This M effect was highly significant. M's abolition of motor seizures was parallelled by dramatic suppression of limbic seizure activity and afterdischarge. The seizure suppressant effect was reversible with a time course parallelling disappearance of stereotypies. Induction of stereotypies with apomorphine did not suppress motor seizure duration. Microinjection of M into several other brain regions did modify the seizures.

We conclude that the ventral midbrain tegmentum, and most likely the SN, is a key site in the propagation of kindled seizure activity triggered by amygdala stimulation. Pharmacologic enhancement of GABA-ergic synapses in this area is an effective method of suppressing these seizures.

24.3 Spontaneous hippocampal interictal spikes: behavioral correlation and effect of scopolamine. L. Stan Leung, Dept. Psychology, University of Western Ontario, London, Canada, N6A 5C2.

Steel wire electrodes were implanted in the CA1 alveus (surface) and the fissure (deep) of both dorsal hippocampi and in the neocortex of rats. Following recording of normal spontaneous activity, each rat was given 10 sec stimulation trains of 50 Hz, 100 - 300 µA, 0.1 msec pulses delivered bipolarly to the right hippocampus. Stimulation trains were given at 1 - 2 hour intervals and not more than 3 times a day. After a few stimulation trains, various abnormal electrical activity could be observed after the high amplitude afterdischarge that immediately followed the stimulation. One example was an increase in fast waves (30 - 70 Hz) by up to 10 fold for a period of 5 - 30 min post-stimulation. By use of Fourier spectral analysis, surface and deep hippocampal fast activities were shown to be highly coherent and 180° phase shifted. After 3 - 10 stimulation trains, spontaneous interictal spikes (SIS) of several mV could be detected visually or discriminated after high-pass filtering. No gross behavioral seizure was observed at this stage. SIS frequency increased with the recovery of the EEG amplitude (1 - 10 min. post-stimulation) and then subsided in one to several hours. SIS were various magnitudes and waveforms but mainly consisted of two types: 1. those with a surface negative, deep positive early phase followed by a late phase of reversed polarity and 2. those with a surface positive, deep negative early phase. Type 1 and 2 correspond to the waveform of the potentials evoked by the stimulation of the basal and apical dendrites of CA1 region respectively (Leung, L. S. Brain Res. 176: 49, 1979). SIS were usually synchronous on both left and right hippocampi. Its frequency varied with the behaviors of the rat, with the highest rate during slow wave sleep (SWS) and the lowest during active movements (walking, rearing, turning) and paradoxical sleep (PS); waking immobility was accompanied by an intermediate SIS rate. Scopolamine (5 mg/kg i.p.) increased the SIS rate across all waking behaviors. SIS were not detected in animals not stimulated by kindling trains.

The variation of the SIS frequency across behaviors resembles the irregular slow activity (ISA) or the theta rhythm of the hippocampus. ISA was largest and theta smallest during SWS when SIS rate was highest; ISA was smallest and theta largest during PS and active movements when SIS rate was lowest. A common mechanism may underlie the generation of hippocampal EEG and SIS - both activities may depend on an ascending brain stem input which includes a muscarinic cholinergic component. Scopolamine increased hippocampal ISA (and reduced relative power of theta) and SIS rate perhaps by blocking the cholinergic input. (Supported by NSERC grants).

24.4 MODULATION OF PENICILLIN-INDUCED INTERICTAL SPIKES BY CHOLINERGIC AGENTS AND MEDIAL SEPTAL LESIONS. Jeffrey H. Goodman* and R.M. Lebovitz. Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

There is strong evidence that the medial septal nucleus (MSN) projects cholinergic fibers, through the fornix that terminate throughout the hippocampus. Acetylcholine (ACH) released from these fibers may act as a neuromodulator of normal pyramidal cell activity. However, acetylcholine's role in the hippocampus during penicillin epilepsy has not been defined.

We used two approaches to investigate the possible role of the septo-hippocampal cholinergic system in penicillin epilepsy. The first was to pharmacologically test the effect of the cholinergic agents, carbachol and physostigmine, on the rate of penicillin-induced interictal spikes (IS). The second was to lesion the MSN and observe the effect on the IS rate.

The left hippocampus of Long-Evans male rats was exposed under Nembutal anesthesia. Sodium penicillin was topically applied to the dorsal surface of the CA1 region of the hippocampus and covered with warmed mineral oil. Once the focus became established, the hippocampus was superfused with artificial cerebrospinal fluid (CSF) and a baseline IS rate determined. The hippocampus was then superfused with the test agent dissolved in CSF. The concentrations of carbachol tested ranged from 10^{-6}M to 10^{-4}M. It was observed that carbachol, in doses of 10^{-5}M and greater, significantly increased the IS rate in a dose-dependent manner. Physostigmine, tested at a dose of 10^{-3}M, also caused a significant increase in the IS rate.

In a separate series of experiments, radiofrequency lesions were placed in the MSN of 300-350 g Long-Evans rats. These animals were allowed to recover a minimum of two weeks at which time the left hippocampus was exposed and penicillin was applied. The exposure was covered with warmed mineral oil. Once interictal spiking began, the spike rate was measured for a period of one hour. At the conclusion of each experiment the brain was removed and the location of the lesion was verified histologically. Non-lesioned controls were surgically prepared and examined in the same manner. We observed that the IS rate in the lesioned animals was significantly slower than the spike rate in the control animals. For example at 30 minutes the mean inter-interictal spike interval for the lesioned animals was 12.77 + 0.72 (SEC $\pm$ SEM) compared to 9.6 $\pm$ 0.56 for the controls ($p<0.005$).

The results suggest that removal of the tonic excitatory cholinergic input to the hippocampus results in a decreased level of excitability within the penicillin focus and that the IS rate can be accelerated by the cholinesterase inhibitor, physostigmine, or by the cholinomimetic carbachol.

24.5 COMPARISON OF IPSP RESPONSES IN THE CONTROL AND KINDLED HIPPOCAMPAL SLICE PREPARATION. M.W. Oliver* and J.J. Miller (SPON: J.P. Pinel). Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Previous studies have suggested that a loss of neuronal inhibition, particularly the inhibitory postsynaptic potentials (IPSP's) mediated by γ-aminobutyric acid, contributes to the generation of epileptiform activity. Although several experimental models have been used to examine this possibility, there is little information available concerning the inhibitory processes present in neuronal tissue predisposed to seizure activity. In the present study, we have utilized the in vitro hippocampal slice preparation to investigate the IPSP's from control and commissural-kindled rats.

Intracellular IPSP's, evoked by stimulation of alveus, were examined in hippocampal CA-1 pyramidal neurons from control and commissural-kindled rats. The potency, resiliency and membrane characteristics of this response was determined by assessing: 1) the duration of inhibition evoked by subthreshold alvear stimulation on spontaneous activity; 2) the effectiveness of the alvear-evoked IPSP to inhibit spontaneously active cells during tetanic stimulation at 5, 10 and 20 Hz; and 3) the conductance changes associated with the IPSP both during and following repetitive alvear stimulation.

The membrane properties of CA-1 neurons were essentially the same in control and kindled slices with their respective values being: RMP, -70, -68 mV; spike amplitude, 96, 91 mV; resting resistance, 27.5, 25.2 MΩ; input resistance during IPSP, 15.8, 18.8 MΩ. Furthermore the duration of inhibition of spontaneous or depolarization-induced neuronal activity ranged from 45-300 msec for controls and 60-350 msec for kindled slices. In both groups, tetanic stimulation at 5, 10 and 20 Hz, for varying durations could result in complete inhibition of spontaneous cellular discharge; interestingly, this occurs when the amplitude of the IPSP is reduced. That is, the amplitude and to a lesser degree the conductance associated with the IPSP are reduced during, but not immediately following, tetanic stimulation. Thus, the apparent effectiveness of the IPSP persists even though its amplitude decreases. Moreover the reduction of the IPSP, irrespective of group, differs at varying frequencies such that the decrease at 5<10<20 Hz.

These data suggest that neuronal tissue predisposed to epileptiform activity maintains the integrity of its inhibitory processes and further that the mechanism(s) subserving kindling and possibly other epileptogenic phenomena are not related to disinhibition, rather, to events that directly promote cellular excitability.

24.6 TRANSFER BETWEEN ELECTRICAL KINDLING OF THE AMYGDALA AND INTRACEREBRAL CARBACHOL OR PENTYLENETETRAZOL. Donald P. Cain. Dept. of Psychology, Univ. of Western Ontario, London, Canada. N6A 5C2.

The repeated administration of a variety of convulsant agents in initially subconvulsant amounts results in the gradual kindling of seizures. We and others have demonstrated that subjects kindled using one convulsant agent can be rendered thereby more susceptible to other such agents, but the mechanism by which this transfer facilitation occurs is not well understood.

Bidirectional transfer has been demonstrated in our laboratory between electrical kindling of the amygdala and peripherally administered pentylenetetrazol (PTZ). However, it was not clear whether kindling with peripherally administered PTZ represented a true CNS kindling effect or was merely an artifact of peripheral administration (changes in peripheral metabolism of the drug), and whether transfer would occur between intracerebrally administered PTZ and electrical kindling. In the present study kindling was clearly demonstrated when generalized seizures gradually developed after approximately 14 bidaily infusions into the rat amygdala of 0.4 mg PTZ in a 1.0 µl volume. Subsequent electrical kindling of the contralateral amygdala through a previously implanted electrode resulted in significantly faster kindling than in a control group. Another group of electrically kindled rats also demonstrated significantly faster chemical kindling when PTZ was subsequently infused into the amygdala. These results demonstrate that intracerebral PTZ can kindle seizures, and that bidirectional transfer occurs between electrical and PTZ kindling.

In a second experiment, transfer between electrical and carbachol kindling of the amygdala was studied. Both a transhemispheric (cannula in one hemisphere, electrode in the other) and an intra-hemispheric test (using a chemitrode) were made. In each case the result was the same: subjects previously kindled with one agent kindled significantly faster with the other agent. Thus, significant bidirectional transfer occurs with these agents. The convulsions produced by the agents were nearly identical. These results indicate that the agents are about equally effective in primary and transfer kindling, and are consistent with the idea that electrical kindling may depend, in part, on activation of muscarinic cholinergic neurons.

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24.7 EFFECTS OF A SINGLE EPINEPHRINE INJECTION ON AMYGDALA SEIZURES AND KINDLING. K.A. Welsh*, L. Kingslow*, and P.E. Gold. Dept. of Psychology, Univ. of VA., Charlottesville, VA., 22901.

Repeated application of electrical stimulation to specific brain areas can lead to progressively intensified EEG and behavioral seizures. This phenomenon, termed "kindling", may be a useful model for studying both epileptogenesis and memory. Because peripheral epinephrine is a potent modulator of memory storage processes, we examined the effects of a single epinephrine injection on the development of kindled seizures.

In male Sprague Dawley rats, electrodes were implanted bilaterally in the basolateral nuclei of the amygdala. One week after surgery, afterdischarge (AD) thresholds were determined using unilateral stimulation trains (monophasic square waves, 1 msec, 60 Hz, 1 sec duration) of ascending intensity. Animals then received IP injections of epinephrine (1.0 mg/kg) or saline and AD thresholds reassessed 30 minutes after injection. AD durations were also measured following a fixed suprathreshold intensity (250 uA) stimulation train. This current intensity was then applied once daily (no further drug injections) and EEG and behavioral seizures were recorded after each train.

Thirty minutes after injection, epinephrine-treated rats had AD thresholds 30% lower than those of controls. Despite this increased sensitivity observed shortly after the epinephrine injection, on the next trial (24 hr later) suprathreshold stimulation (250 uA) resulted in ADs which were shorter (by 50%) in epinephrine-treated rats than in saline controls. Remarkably, the attenuation of AD duration observed after the single epinephrine injection was still evident up to 10 days later; epinephrine-treated animals had shorter ADs during kindling than did saline injected rats. Furthermore, epinephrine-treated rats required fewer trials before exhibiting behavioral clonus than did saline animals.

These results demonstrate several points: 1) A peripheral epinephrine injection reduces the AD threshold in the amygdala. 2) An epinephrine injection reduces the AD duration following suprathreshold amygdala stimulation; this effect is evident long after the injection. 3) A single epinephrine injection is sufficient to attenuate the rate of electrophysiological and behavioral kindling. These central effects of peripheral epinephrine may have potential importance for understanding the mechanisms by which epinephrine modulates memory. In addition, these findings suggest that peripheral adrenergic activity may influence epileptogenesis.

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24.8 NORADRENERGIC ABNORMALITIES IN TWO INDEPENDENTLY DEVELOPED STRAINS OF GENETICALLY EPILEPSY-PRONE RATS. P.C. Jobe, H.E. Laird, T.W. Woods* and J.W. Dailey. Vet. Adm. Med. Ctr. and Depts. of Pharmacology and Psychiatry, LSU Med. Sch., Shreveport, Louisiana 71130 and Dept. of Pharmacology and Toxicology, Univ. of Arizona, Tucson, Arizona 85721.

The seizure prone state of genetically epilepsy-prone rats (GEPR) is characterized by susceptibility to hyperthermic seizures (Jobe et al., Fed. Proc. 41: 1560, 1982) and to sound-induced seizures (Jobe and Laird, Biochem. Pharmacol. 30(23): 3137, 1981). In addition, the GEPR exhibits abnormally low electroshock, pentylenetetrazol and bicuculline seizure thresholds. Two strains of the Sprague-Dawley derived GEPR have been independently developed, one at the Veterans Administration Medical Center in Shreveport, LA and the other at the University of Arizona in Tucson. The Shreveport strain typically exhibits seizures of moderate intensity, whereas the Arizona strain exhibits severe seizures. The Arizona colony was established in about 1957 and the Shreveport colony was independently initiated approximately 16 years later. Originally, seizures exhibited by the Arizona colony were of relatively moderate intensity, overtly identical to those which now characterize the Shreveport colony. Pharmacologic studies support the concept that a noradrenergic deficit may be an etiologically important determinant of seizure susceptibility and intensity in both GEPR strains.

The purpose of the present investigation was to compare innate noradrenergic abnormalities existing within the CNS of both the Tucson and Shreveport colonies. In all CNS areas except the cerebellum, norepinephrine levels were significantly ($P < 0.01$) less in the Shreveport GEPR than in non-epilepsy prone controls. The mean percentage decrement was 24.9%, with a range of 17.9% to 30.9%. The mean decrement in Tucson GEPRs was 37% (Range, 36-39%) with the cerebellum excluded. Norepinephrine levels in the cerebellum were increased by 16% in the Shreveport GEPR, whereas in the Tucson GEPR the levels were decreased by 30%. The existence of norepinephrine decrements which are widespread throughout most of the CNS in both the Shreveport and Tucson GEPRs provide additional support for the concept that one determinant of seizure susceptibility in the two strains in a decrement in noradrenergic transmission. Seizures in the Tucson GEPRs may be more severe than those of the Shreveport GEPRs because of the occurrence of cerebellar differences between these two strains. The increment in norepinephrine levels in the cerebella of Shreveport GEPRs may protect against severe seizures, whereas the norepinephrine decrements in the cerebella of Tucson GEPRs may augment seizure intensity. Alternatively, seizures of Tucson GEPRs may be more severe because of greater norepinephrine decrements in brain areas other than the cerebellum.

24.9 BRAIN NOREPINEPHRINE AND THE GENETICALLY-DIRECTED CONVULSIONS OF THE TOTTERING MUTATION IN THE MOUSE, P.J. Syapin, M. Basura*, S.R Snodgrass, and L. Erinoff*. Dept. of Neurology, University of Southern California School of Medicine, Los Angeles, CA 90033.

The autosomal recessive mutation tottering (tg) is identified by the occurance of "spontaneous" stereotypic behavioral convulsions and a characteristic degree of ataxia. Stereotypic convulsions identical to those seen "spontaneously" are also induced by doses of pentylenetetrazol (PTZ) which are subconvulsant in heterozygote (tg/+) and normal (+/+) mice of the same strain (C57Bl/6J This action of PTZ is antagonized by benzodiazepines but not by other common antiepileptic drugs (Syapin, in press). Levitt and Noebels reported increased brain norepinephrine (NE) levels arising from the locus coeruleus of (tg/tg) mice when compared to (+/+) mice (P.N.A.S., 78:4630, 1981). We now report on the role of the noradrenergic system in "spontaneous" and PTZ-induced stereotypic convulsions of (tg/tg) mice.

Regional brain levels of dopamine, NE, and 5-HT in (tg/tg), (tg/+), and (+/+) mice from our colony are being determined by HPLC to confirm and extend the finding of Levitt and Noebels. To determine whether the noradrenergic system is involved in the expression of stereotypic convulsions, (tg/tg) mice were treated i.p. with α or β-adrenergic drugs 30 min prior to induction of stereotypic convulsions with 30 mg/kg PTZ. Treatment with (+)propranolol (4 mg/kg), phentolamine (10 mg/kg), prazosin-HCl (2 mg/kg), or d-amphetamine (10 mg/kg) had no effect on the induction of convulsions in (tg/tg) mice. Treatment with 1 mg/kg clonidire, however, completely blocked PTZ-induced stereotypic convulsions (N=8). 0.1 mg/kg clonidine was ineffective. Treatment with 1 mg/kg clonidine caused sedation in both (tg/tg) and (tg/+) mice. The protection by clonidine was antagonized by 50% in mice treated with 4 mg/kg yohimbine 15 min prior to clonidine. Alone, yohimbine had only weak anticonvulsant activity.

When forebrain NE was reduced by 80% in adult (tg/tg) mice by i.c.v. injection of 100 µg 6-hydroxydopamine (6OHDA) the ability to have "spontaneous" and PTZ-induced stereotypic convulsions was not changed. Results from an ongoing study show that treatment of 40 day old (tg/tg) mice with 150 µg 6OHDA i.c.v. at least 10 days prior to the onset of behavioral symptoms did not influence the presence of "spontaneous" convulsions or the ataxia used to identify (tg/tg) mice.

These data imply that the noradrenergic system of the (tg/tg) mouse brain is not responsible for the onset of stereotypic convulsions but is perhaps involved in the expression of the behavioral convulsions via α-adrenergic mechanisms. (Supported by a grant from the Epilepsy Foundation of America and grant NS 16314)

24.10 SUBCLINICAL EPILEPTIFORM ACTIVITY AND MORPHOLOGICAL DAMAGE IN THE STRIATUM OF RATS AFTER TRANSIENT FOREBRAIN ISCHEMIA. P.S. Lacy*, W.A. Pulsinelli, Dept. Neurology, Cornell Univ. Med. Coll. New York, N.Y. 10021 (SPON: S.S. Winans)

We have reported that the number of morphologically damaged medium-sized striatal neurons progressively increases for hours after 30 min of severe forebrain ischemia in the rat (Ann Neurol 11:491, 1982). Late changes in arterial blood pressure or oxygenation did not occur in these animals, nor were there signs of postischemic (PI) seizures. We sought to determine (1) if epileptiform activity, defined in this study as spike/sharp-and-slow wavy complex (SW) was present in PI rats without behavioral signs of seizures and (2) if occurrence of SW could be related to the progression of ischemic damage in the striatum.

Tungsten microelectrodes (200 µ) were implanted in the left dorsal-lateral striatum of adult rats 7 to 10 days prior to subjecting the animals to 30 min of severe forebrain ischemia. Forebrain ischemia was produced by occluding the common carotid arteries for 30 min; the vertebral arteries were permanently occluded 24 hr earlier (Stroke 10:267, 1979). The electroencephalogram (EEG) was recorded prior to, during and serially after the ischemic insult. The animals' brains were perfused-fixed with FAM (formaldehyde:methanol:acetic acid, 1:1:8) at 24 to 72 hr after restoration of carotid artery blood flow. Serial sections were examined with the light microscope for ischemic neuronal damage. SW activity was visually identified and defined as spikes (duration < 80 msec) and sharp waves (duration > 80 msec) found to be associated with a slow wave of greater duration. The negative deflection of SW was further characterized according to Ktonas et al. (EEG and Clin. Neurophysiol 51:237, 1981) for amplitudes A and B, and subdurations A and B. On the basis of morphological studies of the striatum, animals were divided into Group I (few neurons damaged, n=3) and Group II (many-majority neurons damaged, n=3). Although SW was arrhythmic, the peak frequency, calculated from 3 min epochs randomly chosen at 30 min intervals, occurred in both groups between 1 to 2 hr PI. The peak frequency in Group I was 28/min and in Group II it was 20/min. The duration of SW from onset at 15 min PI to its termination at 10 hr PI was similar in both groups and was replaced by spindle activity after SW ceased. Amplitude A peaked between 0.5 and 1 hr PI in Group I and between 1 to 2 hr PI in Group II. Amplitude B peaked between 1 to 2 hr PI in Groups I and II. SW could also be detected from other regions of the brain including the neocortex and hippocampus. The results indicate that although striatal SW activity occurred in all rats subjected to forebrain ischemia, there was no apparent correlation between the severity of striatal damage and the frequency or amplitude of SW activity recorded in this region.

24.11 DIFFERENTIAL CONDITIONING EFFECTS OF ENVIRONMENTAL CUES ON KINDLING. F.G. Freeman and P.J. Mikulka*. Psychology Dept., Old Dominion University, Norfolk, VA 23508

Much anecdotal evidence exists suggesting that nonphysiological variables may affect seizures. However, there is relatively little experimental investigation of environmental and behavioral variables which might increase or decrease the probability of seizures. The present experiment investigated the effect of discrimination training on seizures within the kindling paradigm. Male Long-Evans hooded rats were kindled in the amygdala. Group 1 Ss received stimulation in an illuminated chamber with white walls; these Ss were also placed in a darkened chamber with black walls for two minutes either 1/2 hour before or after the kindling stimulation. Group 2 Ss underwent the same treatment except they were kindled in the black chamber and placed but not kindled in the white chamber. Group 3 Ss were kindled in the black chamber and not exposed to the white chamber while Group 4 Ss were kindled in the white chamber and not exposed to the black one. Upon reaching a stage 5 seizure all Ss were threshold tested in both the black and white chamber. They were then given 10 more days of training and retested in chambers.

Subjects kindled in the white box kindled significantly faster than those kindled in the black box ($p < .05$). Subjects in the white-discrimination condition kindled significantly faster than the black discrimination ($p < .01$) while the control groups did not differ. No significant differences in thresholds were found. However, on the final test there was a tendency for the discrimination Ss to have a higher threshold in the box in which they were kindled compared to their "nonkindled" box. Finally, discrimination Ss had significantly shorter AD durations than the control Ss for the threshold tests.

24.12 DIFFERENTIAL RECOVERY OF SIMPLE AND COMPLEX BEHAVIORS FOLLOWING KINDLED SEIZURES IN RATS. S. Caldecott-Hazard, N. Yamagata*, J. Hedlund*, J.C. Liebeskind. Dept. Psychology, University of California, Los Angeles, CA. 90024.

Few studies, either clinical (Escueta et al., 1977; Morrell, 1979) or experimental (Myslobodsky, 1981), have quantitatively evaluated changes in behavior following a seizure. The present study therefore assessed the performance of rats on a variety of behaviors immediately following a kindled seizure and continuing until the behaviors recovered to pre-seizure baselines. Kindling was produced by daily amygdala stimulation until fully generalized (stage 5) seizures were elicited on 3 days. Following a seizure, rats were tested for responses to painful tail shocks, fixed ratio bar pressing for food, free access to food, ability to cling to a vertical grid, latency to first step, and for duration of postictal EEG depression (PID). Each rat was tested on every task, and some rats also received naloxone in order to assess the possible involvement of opioid peptides. One week intervals separated tasks. Tail shocks (100 msec train, 60 Hz, 1 msec square wave pulses, 0.1-10 mA) were given at 10 sec intervals following the cessation of the seizure afterdischarge (AD) and thresholds for a tail flick, single squeak, and multiple squeaks were recorded. Postictally the threshold for multiple squeaks was significantly elevated and remained so for approximately 6 min. Thresholds for tail flick and single squeaks were not significantly changed. Bar pressing for food was abolished for approximately 8 min. Consumption of freely available food was arrested by the seizure but resumed approximately 5 min later. Similarly, the duration of the EEG postictal depression was approximately 5 min. Grid clinging was abolished for approximately 3 min. following the seizure, and the latency to the first step was approximately 2 min. Naloxone pretreatment (10 mg/kg given twice at 15 min. intervals immediately preceding the seizure) shortened the recovery time for the multiple squeak response, for grid clinging, and for the first step. Postictal recovery times for bar pressing, acquisition of freely available food, and PID were not significantly altered by naloxone. These data suggest that simple behaviors such as grid clinging and ambulation recover more quickly following a seizure than do appetitive or pain elicited vocalizations. Complex, learned behaviors such as bar pressing appear to recover most slowly. Furthermore, kindled seizures may release opioids that are involved in the production of analgesia and catatonia, but not in the disruption of learning or food acquisition.

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24.13 KINDLING A SLEEP AND SEIZURE DISORDER. M. N. Shouse* and M. B. Sterman (SPON: D. J. McGinty). Neuropsychology Research, V.A. Medical Center, Sepulveda, CA 91343.

Although prevalent in various epilepsies, sleep pathology is typically viewed as a secondary or incidental complication of seizure activity. We re-evaluated this interpretation by documenting changes in sleep and waking states throughout as well as one month after the development of stimulation induced seizures through basolateral amygdala kindling. Ten cats were experimental subjects. Following recovery from stereotaxic surgery, afterdischarge (AD) thresholds were established using a standard method of limits procedure (Shouse, M. N. and Sterman, M. B., Exp. Neurol., 71: 550, 1982). Stimulation (1 sec, 60 Hz biphasic square waves, 1 msec pulse duration at minimal AD-eliciting intensity) was applied daily until stage 6 generalized tonic-clonic convulsions were obtained. Stage 6 seizure thresholds were established at the end of kindling and after a one month respite from amygdala stimulation, using the same threshold procedure as before. Twelve-hr polygraphic recordings of the sensorimotor cortical electroencephalogram, electrooculogram (eye movements) and electromyogram (neck muscle) were obtained before, during and at the end of kindling as well as on the day preceding seizure threshold determination one month later. An average of 25.6 AD stimulations were required to elicit stage 6 seizures. Progressive slow-wave-sleep and rapid-eye-movement sleep deficits accompanied the development of kindled convulsions, as previously reported (Shouse, M. N. and Sterman, M. B., Exp. Neurol., 71: 563, 1982). Moreover, these deficits persisted one month after kindling procedures were discontinued. Since no overt seizure activity occurred during the interim, non-stimulation period, post-kindling sleep deficits may not be viewed as a simple or transient side effect of seizure pathology. Further, the magnitude of sustained sleep deficits predicted subsequent seizure susceptibility ($r = .82$, $p < .05$). This correlation indicated a more fundamental link between the two pathologies than was previously suspected. Sleep deficits are clearly as persistent as the seizure disorder after kindling, suggesting the "kindling" of a sleep disorder which occurs in addition to and may be a determinant of the seizure disorder.

24.14 LEARNING DEFICITS IN AMYGDALOID-KINDLED JUVENILE RATS. K. J. Ebersole* and R. F. Berman. Department of Psychology, Wayne State University, Detroit, MI 48202.

The present study examined the development and behavioral effects of amygdaloid kindling in juvenile rats. Several lines of research have suggested a relationship between certain types of epilepsy and learning deficits. In particular, infantile seizures (occurring before 2 years of age) are associated with a high rate of mental retardation. An animal model of infantile epilepsy was developed to investigate this problem.

Five groups of young (18-56 days of age) male Long-Evans rats were stereotaxically implanted, bilaterally, in the amygdala with bipolar electrodes 5-7 days prior to the initiation of kindling. Each group differed in the age at which kindling began (25-27, 30-31, 35-36, 40-41, or 49+ days of age). Afterdischarge thresholds (AD) were determined and subjects were unilaterally stimulated once daily (100 Hz, biphasic, square wave, 0.1 msec pulse duration) for 5 sec at threshold until 3 consecutive Stage 5 seizures had been elicited. Development of kindling in juvenile animals was found to be similar to that previously reported for adult rats in spite of extremely high AD thresholds ($\bar{X}$=699 $\pm$ 36 S.E. microamperes) in the young animals. Permanence of kindling was tested 2 weeks to 2 months later and was demonstrated in every animal tested.

Between 12-14 weeks of age 3 groups of animals; a kindled group (n=11), a non-stimulated control group (n=13), and a non-operated control group (n=6), began training on a shock avoidance task. Animals were first water deprived and then trained to run from a start chamber to a goal chamber in which water was available. Training occurred once per day until animals entered the goal box and began drinking in less than 10 sec for 2 consecutive days. There were no significant differences among groups in the number of days required to reach this criterion. The next day each rat was given a brief scrambled footshock (3 milliamperes for 3 sec) upon entering the goal box. Rentention of training was examined 24 hours later by measuring the animal's latency to enter the goal box and begin drinking. The kindled rats showed impaired retention of the shock avoidance training compared to the control groups. Footshock sensitivity was ascertained for all subjects in order to ensure that the observed shock avoidance deficits were not due to an alteration in shock perception. No differences in shock sensitivity were detected among the groups. At the end of the experiments, implanted subjects were sacrificed and precise electrode placements were verified histologically.

These results suggest that while the brains of young rats support kindling, the long range behavioral consequences of this procedure are more profound than those reported for adult kindled animals.

24.15 DEVELOPMENTAL CHANGES IN POST-ICTAL INHIBITION IN THE KINDLED RAT. Bruce J. Albala and Solomon L. Moshé*. Depts. of Psychiatry and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461

Our recent data indicate that amygdaloid kindled rat pups (15-18 days) have faster generalization of focally elicited seizures than adult rats and kindling can be induced in rat pups even after the application of electrical stimuli which either markedly retard or fails to produce kindling in their adult counterparts. This data supports the notion that the immature CNS is more susceptible to the development and initiation of the kindled convulsions, though it does not address any developmental issues once the brain has been "kindled". The present study utilizes the "recycling" technique in an attempt to determine whether the degree of neural maturation affects the extent of post-ictal inhibition.

Fourteen 13 day old rat pups and six 120 day old adult rats were implanted with a bipolar electrode in the left amygdala. Three days later the adults and half the pups were kindled until generalized motor convulsions were elicited. Recycling was used to test the extent of post-ictal inhibition with the delivery of 8 separate stimulations (400 µAmp, 60 Hz, 1 sec) every 2 min. while the depth EEG and behavior was continuously monitored. The electrodes were then removed from the fully kindled (FK) and implanted non-stimulated (NON-STIM) pups which were allowed to grow. At 90 days the surviving rats were reimplanted with a bipolar electrode in the cortex and each amygdala. At 140 days the FK and NON-STIM rats were kindled daily in the right amygdala. The left amygdala was then stimulated until kindled motor convulsions were reliably elicited and the rats were then "recycled" at 5 and 7 months of age.

The results of the first recycling experiment indicates that the rat pups and adults differed significantly for both the behavioral seizure index (pups $\bar{X}$=3.1, adults $\bar{X}$=2.0, $p < 0.05$) and the afterdischarge (AD) seizure index (pups $\bar{X}$=48.8 sec, adults $\bar{X}$=27.5 sec, $p < 0.01$). The surviving 5 FK rats required significantly fewer stimulations to develop generalized convulsions than the 4 surviving NON-STIM rats when first kindled on the right side as adults (FK $\bar{X}$=1.8 stims., NON-STIM $\bar{X}$=5.8 stims., $p < 0.05$). These two groups of kindled animals were not statistically ($p > 0.05$) different in terms of either the first or second adult recycling trial. However, the 4 FK rats that were successfully recycled at 3 different ages had a statistically significant difference ($p < 0.05$) between the behavioral seizure index when they were rat pups ($\bar{X}$=3.2) as compared to their two later adult recycling trials at 5 months ($\bar{X}$=1.7) and 7 months ($\bar{X}$=1.6) of age.

The present study indicates that immature rats are capable of having longer and more severe seizures following delivery of repeated stimuli during the immediate post-ictal period. This increase in post-ictal inhibition with age appears to be a maturational event that is independent of previous epileptic experiences.

24.16 AMYGDALO-THALAMIC INTERRELATIONS IN EXPERIMENTAL TEMPORAL LOBE EPILEPSY. T.E. Hoyt*, P. Rinaldi, J.A. Kusske. Division of Neurosurgery, UCI School of Medicine, Irvine, CA 92714.

The patterns of seizure spread were investigated in rabbits with afterdischarge induced by electrical stimulation of the amygdala. Following surgical procedures, carried out under halothane anesthesia, the animals were immobilized with gallamine triethiodide and ventilated to maintain normal pO_2 and pCO_2. Stereotaxically placed tungsten microelectrode recordings of waves and extracellular unit activity were obtained from the dorsomedial nucleus of the thalamus, both ipsilateral and contralateral to the amygdala being stimulated and from the contralateral amygdala and ipsilateral hippocampus. In one group of rabbits the anterior commissure was left intact. In a second group, prior to inducing afterdischarge by amygdala stimulation, the anterior commissure was electrolytically lesioned. When convulsive activity developed at the focus in preparations with intact anterior commissures there followed a rapid and stereotyped development of ictal activity first detected in the contralateral amygdala and then in the thalamus and finally in the ipsilateral hippocampus. Stereotaxic electrolytic lesions, confirmed histologically, in the anterior commissure were followed by striking disorganization of the spread of ictal activity. In these preparations the duration of seizures was markedly reduced and the amplitude of ictal activity was significantly lessened. The implications of these findings relative to the treatment of patients with intractable temporal lobe seizures may be significant. These studies indicate that further investigations of stereotaxic lesions in the commissural systems connecting limbic structures are warranted to define new approaches to clinical therapy of otherwise intractable seizures arising from the amygdala complex in humans.

24.17 AMYGDALA KINDLING AND NOCTURNAL LOCOMOTION FOLLOWING MIDDLE CEREBRAL ARTERY LIGATION IN THE RAT. J.M. Schwartz*, C.L. Ehlers*, A.J. Mandell, and F.E. Bloom. A.V. Davis Center, The Salk Institute and the Dept. of Psychiatry, UCSD, La Jolla, CA.

Seizures and behavioral deficits are known to occur as sequelae to cerebral cortical infarction in human patients. In the present study we investigated whether alterations in locomotor behavior, and the development of amygdala kindled seizures could be detected following experimental cerebral infarction in the male Wistar rat. Experimental infarction was accomplished under chloral hydrate anesthesia by performing a right (n=12) or left (n=12) fronto-parietal craniotomy and ligating the middle cerebral artery with ophthalamic suture. These ligations resulted in a fronto-parietal cortical lesion 1-3 mm in diameter. Sham rats (n=10) sustained right or left craniotomies and dural puncture. In a second surgical procedure the ligated and sham animals were anesthetized and stereotaxically implanted with electrodes in the amygdala on the side contralateral to the ligation (n=16) or the side ipsilateral (n=8). Two weeks following the surgical procedures locomotion was assessed by monitoring spontaneous nocturnal activity in cages equipped with photocell beams. Ligated and sham rats were tested 30 days post-ligation for threshold to afterdischarge and then kindled daily (1 sec, 60 Hz, bipolar square waves, 200 μA) until 3 fully kindled convulsions were noted.

No gross motor deficits were noted in any of the rats by 72 hours following the ligation. Analysis of nocturnal locomotor activity by ANOVA did not reveal significant group or group x time effects betwen any of the groups (rt vs lf, rt vs shams, lf vs shams, ligated vs shams). However, analysis of the patterns of locomotor activity over time using spectral analysis did uncover significant frequency differences in locomotion. Activity patterns of lesioned animals showed a higher frequency of fluctuation compared to shams irrespective of whether the lesion was on the left or right ($p < .05$ Mann Whitney U-test). Differences in afterdischarge (AD) thresholds and rate of amygdala kindling were also found. Mann Whitney U-test revealed a significant increase in AD threshold ($p < .01$) and kindling rates ($p < .02$) in rats kindled in the amygdala contralateral to the ligation as compared to shams. A non-significant decrease in thresholds and kindling rates was found in animals kindled ipsilateral to the lesion. Thus, it appears that unilateral middle cerebral artery ligation may induce increased inhibitory mechanisms on the contralateral side. These results suggest that cerebral infarction induces complex electrophysiologic and behavioral changes in rats. (Supported by the Klingenstein Foundation and USPHS Grant MH 07899.)

24.18 UNCOUPLING OF HIPPOCAMPAL METABOLISM AND BLOOD-FLOW DURING AMYGDALA-KINDLED SEIZURES IN RATS. R.F. Ackermann, J.L. Lear*, W. Meredith*, and J. Engel, Jr. Reed Neurological Research Center, and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Utilizing the Sokoloff ^{14}C-2-deoxyglucose (2DG) method, we have previously shown that generalized amygdala-kindled seizures are associated with hippocampal hypermetabolism. Those results were replicated in the present study. In addition, two different blood-flow markers, ^{14}C-iodoantipyrene or ^{123}I-N-isopropyl-p-iodoamphetamine (IAM) were administered (i.v.) during, or up to 60 min after, amygdala-kindled seizures. The most salient result was a decrease in hippocampal blood-flow relative to surrounding structures, as indicated independently by both flow markers, during and up to 60 min following the seizures. Several animals with partial seizures (stage 3, Racine) were double-labeled with ^{14}C-2DG and ^{123}IAM. The resulting autoradiographs showed greater 2DG uptake in the ipsilateral hippocampus than the contralateral. In contrast, the IAM autoradiographs from the same animals showed less IAM uptake in the ipsilateral hippocampus than the contralateral. Numerous previous studies have demonstrated global increases in cerebral blood-flow during seizures. The present results suggest that, at the least, such increases are not distributed uniformly; the blood supply to certain structures may actually decrease. Previous workers have attempted to account for hippocampal neuronal loss following prolonged seizure conditions in terms of damage from 'overexcitation', despite the fact that it has been repeatedly shown that 'overexcited' neurons merely cease firing temporarily. The present study's results suggest that a more likely mechanism for progressive hippocampal neuronal death associated with repeated seizures may be that of episodic mismatches between hippocampal metabolic demand and blood supply.

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24.19 RETARDATION OF AMYGDALOID KINDLING IN HOMOZYGOUS DIABETES INSIPIDUS RATS. Bonnie J. Gillis* and Donald P. Cain (SPON: C.H. Vanderwolf) Department of Psychology, University of Western Ontario, London, Ontario N6A 5C2.

There is recent evidence to suggest that arginine vasopressin (AVP) plays a role in CNS functioning. Several investigators have demonstrated that AVP fibres project from the hypothalamus to many parts of the brain including medial, central, lateral and basal amygdala, and that electrical stimulation of the amygdala results in increased levels of AVP at the site of stimulation. Kasting et al (1980) described a sensitization process whereby intracerebral ventricular injections of AVP initially caused short periods of immobility, and myoclonic-myotonic convulsions after a second injection two days later. The present study examined electrical kindling of the amygdala in homozygous diabetes insipidus rats, a Long Evans strain that genetically lacks the ability to synthesize AVP. It is of theoretical interest and of clinical significance to determine whether AVP plays a role in the production of generalized seizures.

Adult male homozygous diabetes insipidus (DI) and normal Long Evans (N-LE) rats were implanted with bilateral electrodes in the basolateral amygdala. Kindling stimulation consisted of a 1-sec train of biphasic square wave pulses at 60 Hz delivered once a day at 200 μA. The N-LE subjects kindled to a stage 5 generalized seizure after a mean of 14.0 afterdischarges (ADs). After 20 ADs the DI subjects had progressed to a mean seizure stage 1 (immobility and chewing). Half of these subjects were continued on the 200 μA stimulation and they kindled after a mean of 28.7 ADs. The remaining DI subjects were stimulated at 400 μA for 3 d., followed by stimulation at 800 μA for 3 d., after which the experiment was terminated. These latter DI subjects progressed to a mean seizure stage 1 after 26 ADs. Thus, the rate of kindling in the DI subjects was significantly retarded relative to that of the N-LEs ($p < .01$). There was no difference between the DI and N-LE subjects with respect to AD threshold or generalized seizure threshold (minimum current intensity necessary to elicit a generalized seizure in a fully kindled rat) in those subjects that kindled.

These preliminary data suggest that AVP may play a role in the kindling of seizures from the amygdala of normal LE rats, and that the significant retardation of kindling in the DI subjects may have been due to their lack of AVP. A variety of studies are being conducted to test this hypothesis more directly.

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24.20 ROLE OF CENTRENCEPHALIC SYSTEM IN EPILEPSY. G.H. Fromm, C.F. Terrence* and A.S. Chattha*. Dept. of Neurology, Univ. of Pittsburgh Sch. of Med. and Neurology Service, V.A. Hospital, Pittsburgh, PA 15261.

It is now generally accepted that seizures originate in the cerebral cortex rather than in the centrencephalic system. However, there continues to be controversy as to whether seizure activity spreads primarily via the corpus callosum and cerebral commissures or via the centrencephalic system (reticular formation of diencephalon and midbrain). A number of investigations have indicated that anticonvulsants prevent the spread of seizures through subcortical pathways, suggesting that these structures do play an important role in seizure spread and generalization.

We have found the various inhibitory and excitatory mechanisms in the trigeminal nucleus of cats to be a useful model for studying the mechanism of action of anticonvulsant drugs. Petit mal drugs selectively depress inhibitory mechanisms in this model, while grand mal drugs primarily depress excitatory mechanisms. We have now compared the effect of anticonvulsant and non-anticonvulsant drugs on the periaqueductal and periventricular inhibition of the trigeminal nucleus to further elucidate the role of the reticular formation in the spread and in the control of seizure activity.

The i.v. injection of 5-20 mg/kg carbamazepine depressed the periventricular and the periaqueductal inhibition to the same extent that it depressed excitatory transmission in the trigeminal nucleus. As in previous experiments, these doses of carbamazepine facilitated the segmental inhibition in the trigeminal nucleus. The i.v. injection of 0.5-2.0 mg/kg baclofen also depressed excitatory transmission in the trigeminal nucleus, but in contrast to carbamazepine facilitated the periventricular and periaqueductal inhibition, as well as the segmental inhibition.

Baclofen thus resembles carbamazepine in depressing excitatory transmission and facilitating the segmental inhibition. Baclofen also resembles carbamazepine in being an effective drug for the treatment of trigeminal neuralgia. On the other hand, baclofen does not depress the periventricular and periaqueductal inhibition, and it does not have anticonvulsant properties either. Our experiments therefore indicate that the ability to depress the reticular formation is an important characteristic of anticonvulsant drugs. This suggests that the centrencephalic system is indeed involved in the spread and generalization of seizures.

25.1 THE EFFECT OF IMMOBILIZATION STRESS AND OPIATE RECEPTOR BLOCKADE ON RAT SUBSTANTIA GELATINOSA ACTIVITY DEMONSTRATED WITH CYTOCHROME OXIDASE HISTOCHEMISTRY. G.S. Hamill and N.J. Lenn. Depts. of Neurol. and Pediat., and Clinical Neurosci. Research Center., University of Virginia, Charlottesville, VA 22908.

The substantia gelatinosa (SG) is a complex area of interaction between primary afferents, projection cell dendrites, spinal interneurons, and descending pathways. Zeiss Zonax microdensitometer scans of cytochrome oxidase histochemical reaction (COx) in cervical spinal cord sections were corrected for staining variations based on dorsal funiculus, and control SG values amongst batches. Twelve rats were stressed by immobilization (IS) for one hr between 0900 and 1500 daily for 2 weeks; 5 of these also received naloxone HCl (Nal) in increasing dosage of 0.27 to 0.40 mg/kg s.c. 3 times daily. Five rats not immobilized received standard care, and 2 received naloxazone (nal) 20 mg/kg i.v. alternate days for 4 doses.

Visually apparent differences in staining intensity amongst the 4 experimental groups were confined to SG. The control animals had a mean COx density in SG of 360±28, while daily IS resulted in a 53% increase to 550±51 ($p<.02$). IS+Nal resulted in COx 29% less than IS only, 390±32 ($p<.05$), not significantly different than controls ($p>.2$). Nal was similar to control. IS+Nal rats were docile when handled compared to IS only.

There is evidence that COx is proportional to neuronal activity, analogous to 2-DG uptake, but with a time constant of days to weeks. The increased COx with IS in the present study might result from the effect of IS on brain. However, the effect of IS on brain is enhanced by Nal and attenuated by morphine (Nal reversible). In contrast Nal reverses the effect of IS on SG activity, suggesting a direct effect of IS and/or Nal on SG. Furthermore that IS gives greater COx than control, IS+Nal and nal only suggests that Nal interacts with the IS effect, possibly at a common site. Since these are net measures of activity direct effects on SG, and via multiple brain sites cannot be further distinguished, and very different component contributions of several sites may result in similar values.

In addition to providing evidence for interactive effects of IS and opiates on SG activity, we have provided further support for the use of COx for spatial resolution of physiological and pharmacological alteration of neuronal activity. Supported by NIH grant NS 16882. Naloxazone courtesy of Dr. G. Pasternak.

25.2 ROLE OF PEPTIDES, SUBSTANCE P AND ENKEPHALINS, IN ACUPUNCTURE ANALGESIA: IMMUNOCYTOCHEMICAL STUDY IN RAT SPINAL CORD. L. L. Vacca, N. Eric Naftchi, Guan Xinmin*, and Ai Minkang*. Department of Anatomy, University of Kansas Health Science Center, Kansas City, Kansas 66103, Laboratory of Biochemical Pharmacology, N.Y.U. Med. Ctr., New York, NY 10016, and Acupuncture Anesthesia Research Department, Wuhan Medical College, The People's Republic of China*.

Using immunocytochemistry, we are investigating the effect of acupuncture on the peptides substance P (SP) and methionine and leucine enkephalins (ME and LE, respectively) in the rat spinal cord. The peptides are thought to play a role in spinal pain processing. That is, SP seems to be involved in nociception, and the enkephalins, which bind stereospecifically to opiate receptors, have a role in analgesia. Our experiments so far show that acupuncture, like morphine (Vacca, L.L., Abrahams, S.J., Naftchi, N.E., Brain Research, 182:229, 1980), causes the increase of intraneuronal SP in the dorsal horn substantia gelatinosa of the rat spinal cord. The SP increase varies in proportion to the increase in pain threshold after acupuncture. Treated rats which show a great increase in pain threshold also exhibit a dramatic increase in SP; treated rats with a smaller increase in pain threshold after acupuncture exhibit little to no increase in SP.

Among treated animals, immunologic staining for ME varies according to the antisera species. Slight reductions appear variably in the dorsal horn of treated rats which show a great increase in pain threshold; no change occurs when acupuncture causes a smaller increase in pain threshold.

Compared with ME, LE exhibits greater reductions in the dorsal horn after acupuncture. However, once again the results vary between experiments, and change in proportion to the increase in pain threshold after acupuncture.

Despite the variations, we interpret the data to mean that acupuncture may cause the release of LE and may also block the release of SP from nearby afferent processes. Current hypotheses suggest enkephalin interneurons synapse with the preterminal endings of SP afferents and regulate SP release through an interaction with the opiate receptor. Conceivably acupuncture first causes the release of LE which then binds to preterminal opiate receptors and subsequently blocks SP release. Using our antisera species, ME does not change dramatically. Therefore LE and ME may have different functions and responses in vivo.

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25.3 INTRATHECAL DYNORPHIN INDUCES ANTINOCICEPTION AND PARALYSIS. B.H. Herman. Addiction Research Foundation, 701 Welch Road, Palo Alto, CA 94304.

Previously, we found that intraventricular administration of the partially sequenced dynorphin peptide (dynorphin-(1-13) induced antinociception in rats (Herman, B.H., Leslie, F. & Goldstein, A., Life Sci., 27: 883, 1980). In contrast with other opioids, high naloxone doses were required to antagonize these effects. These results as well as others have suggested that dynorphin may preferentially interact with a unique type of opiate receptor in brain.

Here we determined if intrathecal (IT) administration of dynorphin induces antinociception in rats. We also compared dynorphin with morphine (a mu ligand) and (D-Ala2, Leu5) enkephalin (DADLE, a delta ligand) to determine the type of opiate receptor that dynorphan interacts with in spinal cord.

IT dynorphin (ED_{50} = 6.16 nmol, N = 9) was 14 times less potent than morphine (ED_{50} = 0.44 nmol, N = 9) and 103 times less potent than DADLE (ED_{50} = 0.06 nmol, N = 11) in the tail flick test (50% maximum possible effect). On hindlimb clip vocalization and flexion tests, antinociceptive potency of the three drugs were similar, although ED_{50}s were orders of magnitude higher than in the tail flick test. In these tests, the potency of dynorphin-(1-13) was similar to dynorphin while (Leu)enkephalin produced no effect in doses up to 50 nmol.

IT dynorphin doses greater than 10 nmol also induced hindlimb paralysis. Such paralysis was not reversible if the dynorphin dose was greater than 20 nmol. Neither IT morphine or DADLE produced similar paralysis, although doses of DADLE that were 100 times the ED_{50} for tail flick did induce a waxy flexibility of the hindlimbs.

Naloxone (1 mg/kg, s.c.) completely antagonized the antinociceptive effects of morphine (0.88 nmol) and DADLE (0.12 nmol) but not dynorphin (6.00 nmol) on tail flick. High naloxone doses (32 mg/kg, s.c.) did antagonize dynorphin's effects.

These results suggest that in spinal cord, dynorphin may have a role in antinociception and in some aspect of motor function. These data also raise the possibility that more than one opiate receptor type may modulate pain in spinal cord.

25.4 MODULATION OF THE SOMATOSENSORY RESPONSES OF SPINAL CORD DORSAL HORN NEURONS IN THE CAT BY ELECTRICAL STIMULATION OF THE RED NUCLEUS. Jonathan O. Dostrovsky and Bruce G. Gray. (SPON: H.C. Kwan). Department of Physiology, University of Toronto, Toronto, Canada, M5S 1A8.

Previous studies on the role of the red nucleus in descending control systems have found that this structure is important in modulating motor related responses. However anatomical studies have shown that the rubrospinal tract terminates in laminae V-VII, a region also known to contain neurons which relay somatosensory information. Such a pattern of termination would seem suited for modulating dorsal horn neurons which respond to cutaneous stimuli. Thus it was decided to investigate the effects of electrical stimulation delivered to the red nucleus on the responses of neurons receiving somatosensory inputs.

Experiments were performed on chloralose anesthetized cats. Single unit recordings were obtained from the lumbar spinal cord using glass-coated platinum-plated tungsten microelectrodes. A bipolar stimulating electrode was stereotaxically implanted in the red nucleus and the stimulation site subsequently verified histologically. A total of 26 neurons was studied. These neurons were identified according to standard criteria as low threshold mechanoreceptive, wide dynamic range or nociceptive specific by a careful sensory examination. Most of these neurons were located in laminae IV and V of the dorsal horn. To test for inhibitory effects from the brainstem the neurons were excited at just suprathreshold levels by electrical skin stimulation applied to the cutaneous receptive field. The brainstem conditioning stimulus was delivered 130 ms prior to the peripheral stimulus and consisted of a 100 ms 500 Hz train of 0.1 ms pulses (maximum current 400uA). 10/14 low threshold mechanoreceptive, 9/10 wide dynamic range and 2/2 nociceptive specific cells were inhibited with mean currents of 104uA, 121uA and 59uA repectively. A preliminary analysis of stimulation sites in or around the red nucleus revealed that those placed more caudally were most effective. These results indicate that the red nucleus may exert some modulatory influence on the transmission of somatosensory information in addition to its role in motor control. The inhibitory effects are presumably mediated by the rubrospinal tract, although the involvement of the rubro-bulbospinal pathway whose spinal component is thought to be the dorsal reticulospinal tract can not be ruled out.

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25.5 ASCENDING AND DESCENDING PROJECTIONS OF N.R. MAGNOCELLULARIS (NMC) AND N.R. GIGANTOCELLULARIS (NGC): AN AUTORADIOGRAPHIC AND HRP STUDY. Frank P. Zemlan, Michael Behbehani and Robert M. Beckstead. Psychobiology Division and Dept. of Physiology, Univ. of Cin. Sch. of Med., Cincinnati, OH 45267 and Dept. of Anatomy, Univ. of Va. Sch. of Med., Charolettesville, VA 22908.

The localization and density distribution of NMC and NGC cell bodies projecting to the spinal cord were determined in HRP experiments in the rat. Spinal cord hemisections with subsequent HRP application were performed at C_4 and T_{10}. The greatest density of NGC HRP labeled cells was found in the dorsorostral extent of NGC, while the greatest density of NMC HRP labeled cells was observed in caudal NMC. This seperation of NGC- and NMC-spinal neurons allowed highly selective labeling of these two systems in autoradiogrpahic experiments. Microiontophoretic deposits of ^{3}H-amino acids were made in the dorsorostral aspect of NGC in the rostral medulla, while NMC deposits were made in the caudal aspect of NMC in the caudal medulla.

Descending NGC projections were observed to the ipsilateral lateral vestibular nucleus, n. VII, XII; the contralateral inferior olive and ventral aspect on n. cuneatus. At cervical levels NGC-spinal fibers coursed through the ventral columns bilaterally with projections to laminae VII, VIII and X. Ascending NGC projections were observed to the ventral aspect of the periaqueductal gray, largely excluding raphe dorsalis; the deep and intermediate layers of the superior colliculus, the intralaminar nuclei, Fields of Forel and the dorsal and lateral hypothalamic nuclei.

Descending projections from NMC were observed to the lateral reticular nucleus, d.m.n. X, n. XII, n. commisuralis and intercalatus. At cervical levels NMC fibers coursed through the ipsilateral lateral columns, and were most numerous dorsally. Projections were observed to laminae IV, V, VI ipsilaterally, and bilaterally to VII and VIII. A similar pattern of labeled fibers was observered at caudal levels of the cord including a projection to the ipsilateral intermediolateral columns. No projection to the substania gelatinosa was observed after either microiontophoretic or large pressure injections of label to NMC. Although substantial descending projections were found with small microiontophoretic injections of label to NMC; ascending projections were limited to the ventromedial aspect of the pontine reticular formation. When large pressure injections were made to NMC, which spread into NGC, ascending projections were similar to those observed after NGC injections, and similar to those reported by others for the cat and opposum employing pressure injections.

25.6 CATEGORIES OF AXONS IN VENTRAL ROOTS OF THE CAT. B. Ferguson and D. Emery. Dept. of Zoology, Iowa State University, Ames, IA 50011

Unmyelinated axons in human ventral spinal roots are distributed uniformly throughout all spinal segments (Coggeshall et al., Brain 98:157-166. 1975.) In contrast, ventral roots in the rat (Coggeshall et al., J. Comp. Neurol. 173:175-184. 1977) and frog (Vance et al., J. Comp. Neurol. 164:117-126. 1975) show a variable distribution of unmyelinated fibers with some spinal segments containing large populations of unmyelinated axons and other spinal segments containing very few unmyelinated axons. One of the purposes of this study was to determine if the distribution of unmyelinated fibers in ventral roots of the cat is variable as in the frog and rat or uniform as in human ventral roots.

Ventral roots L_1-L_6 of the cat were examined with the electron microscope to determine the numbers of myelinated and unmyelinated fibers. All lumbar ventral roots contain sizeable populations of unmyelinated axons (Table 1). Levels L_1-L_4, which are part of the visceral outflows contain large populations of small myelinated fibers which are believed to be preganglionic. However, levels L_5 and L_6 do not contain large populations of small myelinated fibers, supporting the assertion that these segments in the cat are between the visceral outflows. Thus, the large populations of unmyelinated axons in L_5 and L_6 ventral roots are not preganglionic fibers.

Counts of axons after ventral rhizotomies and dorsal root ganglionectomies showed that most of the unmyelinated axons in ventral root L_6 are of dorsal root ganglion origin. These results are similar to findings by other workers that ventral roots between the autonomic outflows contain primarily unmyelinated sensory and myelinated efferent fibers. The distribution of unmyelinated axons in ventral roots of the cat is similar to the uniform distribution of unmyelinated axons in humans, but differs from the variable distribution of unmyelinated axons in the rat and frog.

The number and percentages of myelinated and unmyelinated fibers in ventral roots of the cat are presented below:

Table 1. Lumbar spinal segments

	Myelinated	Unmyelinated
L_1	2142(82.1%)	466(17.9%)
L_2	3106(82.5%)	657(17.5%)
L_3	2699(78.6%)	736(21.4%)
L_4	3115(71.4%)	1249(28.6%)
L_5	4321(71.4%)	1733(28.6%)
L_6	4819(68.7%)	2194(31.3%)

Table 2. Ventral root L_6

Cat	Myelinated	Unmyelinated
2	4819(68.7%)	2194(31.3%)
4	5657(77.4%)	1648(22.6%)
6	4704(71.7%)	1861(28.3%)
7	4530(82.6%)	952(17.4%)
8	5445(79.7%)	1384(20.3%)
9	3608(76.8%)	1093(23.2%)
	$\bar{X}$=4794(75.9%)	$\bar{X}$=1522(24.1%)

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25.7 THE PRE AND POSTNATAL DEVELOPMENT OF FLAME-SHAPED HAIR FOLLICLE PRIMARY AFFERENTS IN THE RAT DORSAL HORN: A GOLGI STUDY. J.A. Beal. Dept. of Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130.

Primary afferent collaterals which enter the head of the dorsal horn from its ventral aspect have been described in newborn animals as the "large" or "deep" fibers (Ramon y Cajal, S., Histologie du Systeme Nerveux, 1909) and as "flame-shaped" arbors (Scheibel, M.E. and A.B. Scheibel, Brain Res. 9:32-58, 1968). These same afferents have been identified in the adult and physiologically characterized as A-delta hair follicle afferents (A.G. Brown et al., J. Physiol. 272:779:797, 1977). Some structural differences, however, between the newborn and adult patterns have been noted. Studies on the newborn have demonstrated that "flame-arbors" form narrow lobules which extend through both the inner and outer zones of the substantia gelatinosa (SG) and that some give rise to collaterals to more than one lobule. Studies in the adult, on the other hand, have shown that these afferents extend dorsally no farther than the inner zone of the SG and have no interlobular collaterals. These discrepancies prompted the present study on the development of the "flame-shaped" hair follicle afferents. Sequential stages at close intervals were examined from 15 days gestation to 20 days post-partum. The hair follicle afferents can first be identified at 19 days of gestation. In transverse sections at this stage, small caliber fibers are seen leaving the dorsal funiculus in an arched pattern passing down into the neck then swinging upward into the head of the dorsal horn. At this stage the afferents have few branches and extend dorsally no farther than lamina III. Mechanisms directing the growth of these afferents is not yet clear but it has been observed that the growing afferents closely follow the vascular pattern and may be growing into spaces provided by regressing glial processes. At birth, the afferent arbors begin to branch and take on the characteristic flame-shaped lobular appearance. In addition to dorsal branches which by now enter the inner zone of the SG, many arbors also give rise to short ventral twigs which extend deep into lamina IV several micra below the arch of the incoming afferent fiber. By 10 days post-partum the hair follicle afferents have nearly attained their adult appearance. A few afferents at this and later stages have one or two branches which enter the outer zone of the SG, but these represent only a small percentage. In no case did branches of the hair follicle afferents enter more than one lobule. Thus, the integrity of each afferent lobule is established early in development. (Supported by NIH Grant #NS16642-02.)

25.8 DEVELOPMENT AND MATURATION OF NEURONS IN THE SUBSTANTIA GELATINOSA (SG) OF THE RAT SPINAL CORD: A GOLGI AND ELECTRON MICROSCOPIC STUDY. H. Ryan Bicknell* and John A. Beal (SPON: J.E. Penny), Dept. of Anatomy, Louisiana State Univ. Medical Center, Shreveport, LA 71130.

Although a considerable amount of data has been amassed, the developmental pattern of the SG is not clear. Ramon y Cajal (Histologie du Systeme Nerveux, 1909) followed the development of SG neurons and described the early prenatal formation of three general cell types ("centrale", "limitrophe", and "transversaux" cells) which display long axons and lengthy, but relatively simple dendritic arbors. In early postnatal material, however, Cajal noted drastic changes in the appearance of the cells. Axons now had collaterals but could no longer be followed into the white matter and the number of drendrites had increased, yet they were shorter and covered with numerous "fine filaments".

In order to further elucidate and more precisely describe these developmental changes, the present study was initiated. SG neurons in the rat lumbar spinal cord were examined at short-intervaled sequential stages from 15 days of gestation to 20 days post-partum. Our results suggest that Cajal's pre- and postnatal descriptions involve two separate populations of developing cells rather than variations within the same population. Using the Golgi technique, most cells in prenatal developmental periods appear to be "centrale", "limitrophe", or "transversaux" cells with long axons. These neurons have relatively simple dendritic arbors which lengthen throughout the developmental period and are thought to be Golgi type I projection or propriospinal neurons. Postnatally, a distinctly different group of cells begin to develop. These neurons develop several collaterals and display numerous short radiating dendrites. Close examination of various stages of development indicate that these neurons undergo a gradual dendritic metamorphosis and develop into an assortment of various Golgi type II interneurons. Both light and electron microscopic data suggest that these interneurons represent a large population of cells as indicated by a marked increase in the mean size of neuron cell bodies and an increase in the number of small diameter axons and dendrites during the postnatal developmental period.

In conclusion, the present study shows that SG neurons are heterogeneous and develop in two major waves. Golgi type I neurons essentially mature prenatally while Golgi type II interneurons mature postnatally, and make up the largest portion of the SG population.

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25.9 ELECTRON MICROSCOPIC ANALYSIS OF PRIMARY AFFERENT FIBER TERMINALS FROM SLOWLY ADAPTING (TYPE I) RECEPTORS. K. Semba, P. Masarachia*, S. Malamed*, M. Jacquin, S. Harris*, G. Yang* and M. D. Egger. Dept. of Anat., UMD-Rutgers Med. Sch., Piscataway, NJ 08854.

Using the intra-axonal horseradish peroxidase (HRP) technique, we have previously described characteristics of terminals in the dorsal horn of primary afferent fibers innervating Pacinian corpuscles (PAC) in the glabrous skin of the cat's hindpaw (Semba et al., Neurosci. Abstr., 7(1981):528). The aim of the present study was to analyze terminals from another type of low-threshold mechanoreceptor, i.e., slowly adapting (SA) Type I receptors, and to compare these with PAC afferent terminals.

Analysis of electron micrographs from Rexed's laminae IV-VI indicated that: (1) HRP labelled boutons contained clear, round synaptic vesicles. Their longest dimensions ranged from 0.9-2.9 μm, somewhat less than those of PAC afferent terminals. About 13% of the perimeter was devoted to synaptic specializations. (2) About 70% of the synaptic contacts made by labelled boutons were with dendrites, about half on spines and half on shafts. Unlike PAC afferent terminals, contacts were frequently seen on dendrites near the soma. We observed no axosomatic contacts. Ninety percent of axospinous and axodendritic contacts were asymmetrical; subsynaptic bodies were often visible postsynaptically. Some postsynaptic dendritic spines contained a few dense core vesicles. We observed one spine with pleomorphic vesicles apparently presynaptic to a labelled bouton. (3) The remaining 30% of the synaptic contacts were with unlabelled axons. Most of these were postsynaptic to the labelled boutons. In 70% of these postsynaptic unlabelled axons, postsynaptic densities characteristic of asymmetrical synapses were visible. We also observed several unlabelled axons apparently presynaptic to labelled boutons, one of which formed a reciprocal synapse with a labelled bouton. (4) Lengths of synaptic specializations with axons (0.12-0.56 μm) tended to be less than those with dendritic spines (0.17-0.93 μm) or with dendrites proper (0.18-0.78 μm). (5) Labelled boutons often formed glomeruli involving several structures, some of which in turn were synaptically interrelated. (6) We found no systematic differences in the morphology of the labelled boutons among laminae IV-VI.

The predominance of synapses on dendrites, some at their proximal portions, combined with the presence of clear, round vesicles in the labelled boutons, suggests that impulses from SA receptors in the glabrous skin of the cat's hindpaw produce strong excitation of dorsal horn cells.

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25.10 ANATOMICAL STUDIES OF LAMINA X IN THE RAT. R.L. Nahin and G.J. Giesler, Jr., Dept. of Neuroscience, University of California, Los Angeles, CA. 90024 and Dept. of Anatomy, University of Minnesota, Minneapolis, 55455.

Previous studies of Lamina X have not provided a reliable quantitative description of its lateral boundary. Using Nissl-stained material from the rat spinal cord, measurements of neuronal size, type, and density were obtained for several regions of the gray matter surrounding the central canal. Neuronal packing density was found to be relatively uniform throughout Lamina X. However, within a region of 190-260 microns (mean=232.6, S.D.=24.0) from the center of the central canal in the lumbar enlargement, an abrupt increase in cell density and cell size was observed. We believe these differences comprise a useful definition for the lateral boundaries of Lamina X. In a similar manner, boundaries of Lamina X in the cervical enlargement (mean=293.4, S.D.=23.5) and the mid-thoracic spinal cord (mean=184.1, S.D.=16.8) were determined. The vast majority of neural peptides and opiate receptors known to be present in the area around the central canal are confined to Lamina X as defined here.

The ascending projections of Lamina X neurons were studied by use of the retrograde transport of horseradish peroxidase (HRP). Predominately contralateral labeling of Lamina X neurons appeared following large injections of HRP into the medulla, pons and midbrain. Large numbers of cells within Lamina X were labeled following HRP injections into the medullary reticular formation. Far fewer cells were labeled with injections into the pons or midbrain. Labeled cells were categorized according to their shape as pyramidal, stellate or fusiform. For all injections stellate cells were the most frequently encountered. Pyramidal and stellate cells were distributed uniformly throughout Lamina X. Fusiform cells were found predominently in the dorsal-most region of Lamina X. Following medullary and pontine injections, Lamina X cells labeled in the lumbar enlargement were significantly larger than those labeled in the cervical enlargement. This difference was apparent across all three cell types. These data suggest Lamina X neurons not only contribute to local nociceptive processing as indicated by Honda and Perl (1981), but also contribute significantly to long ascending pathways including the spinoreticular tract. (Supported by NIH grants NS07628 and NS17540.)

25.11 IMMUNOCYTOCHEMICAL ANALYSIS OF SEROTONIN AXONS IN LAMINAE I AND II OF THE LUMBAR SPINAL CORD OF THE CAT. M.A. Ruda, J. Coffield*, and H. Steinbusch*[1]. Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205; [1]Free University, Amsterdam, The Netherlands.

Serotonin (5HT), a monoamine neurotransmitter originating from brain stem cell groups, is in part responsible for descending modulation of the response of dorsal horn neurons to noxious input. To identify the site of action of 5HT in the dorsal horn, we employed the PAP immunocytochemical technique of Sternberger to characterize 5HT axons and terminals at the light (LM) and electron microscopic (EM) level. Adult cats were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. Glutaraldehyde (0.2%) was added to the EM perfusate. Fifty μm transverse and sagittal sections were cut with a Vibratome. For LM, 0.75% Triton X100 was added to the 1^{o}, 2^{o} and PAP to improve penetration while no detergents were added to the EM material. The characteristics of the 5HT antisera were as previously described (Steinbusch et al., Neurosci., 3 (1978) 811).

Immunoreactive 5HT axons were most numerous in lamina I, decreased somewhat in lamina IIa and were fewest in lamina IIb. In laminae I and II, the predominant orientation of the varicose 5HT axons was rostro-caudal. An occasional branching of the varicose strand was found. At the LM level, at least three different types of immunoreactive axons could be determined based on the size of their varicosities. At the EM level, 5HT endings synapsed primarily on dendritic shafts, and occasionally on spines and cell bodies in both laminae I and II. Dendrites which received 5HT synapses varied with respect to their cytoplasmic density and the presence or absence of vesicles. Most synapses were slightly asymmetrical with only a slight accumulation of dense material on the postsynaptic side. Immunoreactive 5HT endings were never observed as part of axoaxonic synapses. The 5HT varicosities contained either a mixture of flattened and small oval agranular vesicles, or a relatively homogeneous population of oval vesicles. A few dense core vesicles were present in most 5HT endings. The PAP reaction product coated the agranular vesicles, dense core vesicles and outer mitochondrial membranes. Since 5HT endings synapsed on cell bodies in laminae I and II as well as on different morphological types of dendrites, it is likely that 5HT modulates the response properties of different types of neurons in the superficial dorsal horn. Additionally, the presence of numerous 5HT synaptic contacts on dorsal horn neurons suggests that 5HT modulation of nociceptive information occurs predominantly through synapses on dorsal horn neurons themselves, rather than on incoming primary afferent axons.

25.12 SEROTONERGIC INNERVATION OF DORSAL COLUMN POSTSYNAPTIC SPINOMEDULLARY (DCPS) NEURONS IN THE CAT AND MONKEY. N. Nishikawa*, M.A. Ruda, G.J. Bennett and R. Dubner (SPON: R. Dionne). Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

Bulbospinal serotonergic (5HT) neurons are known to modulate the activity of spinal cord projection neurons. It is unclear whether this modulation is presynaptic, postsynaptic, or both. It has recently been shown that DCPS neurons in cats and monkeys constitute a major somatosensory projection that is as large as the feline spinocervical system. We have examined the 5HT innervation of DCPS neurons with a combined retrograde labeling and immunocytochemical method.

Lumbosacral DCPS neurons were retrogradely labeled by placing HRP on their cut axons in the thoracic dorsal columns. Retrogradely transported HRP was developed with $CoCl_2$-DAB to yield a reaction product consisting of black granules dispersed throughout the cell's soma and proximal dendrites. The tissue was subsequently counterstained with an anti-5HT antibody by the PAP method. 5HT profiles were stained an homogenous reddish brown. Immunocytochemical controls were all negative.

In the cat, 5HT varicosities were present in great numbers throughout the grey matter. Retrogradely labeled DCPS neurons were found in laminae IV, medial V-VI, and dorsomedial VII. Every DCPS neuron was surrounded by 5HT varicosities that appeared to make direct contact with the cell. Counts of the varicosities impinging on 20 consecutive DCPS neurons showed an average of 61 contacts per cell.

In the monkey, 5HT varicosities were far less numerous than in the cat. DCPS neurons were concentrated in laminae III-IV and scattered in laminae V, VI, VII, and X and also in the dorsolateral white matter. Almost all of the monkey's DCPS neurons had 5HT varicosities contacting their perikarya or proximal dendrites. These contacts were generally not as numerous as in the cat. An average of 18 contacts per cell was found on 20 consecutive DCPS neurons.

These light-microscopic observations indicate that the cat's and monkey's DCPS projections are modulated by bulbospinal 5HT systems and that this control is at least partly postsynaptic.

25.13 ELECTRICAL ACTIVITY AND NEUROPEPTIDE LOCALIZATION IN THE LUMBAR DORSAL HORN AFTER DORSAL ROOT AVULSION AND RHIZOTOMY IN THE CAT. J. Ovelmen Levitt*, B. Blumenkopf*, R. Sharpe*, K. H. Lee* and B. S. Nashold. Div. Neurosurgery, Duke Un. Med. Ctr., Durham, N. C. 27710

A feline model of lumbar plexus avulsion has been developed with unilateral extradural avulsions of 4-5 roots of the lumbar enlargement performed. These preparations were studied acutely and up to 6 months post-denervation. Dorsal rhizotomized animals were also studied and experimental results compared. Electrophysiological experiments were performed under light barbiturate anesthesia. In chronic denervations there was an increased amplitude and duration, although prolonged in latency, of the response to electrical stimulation of receptive areas of adjacent intact segments into the denervated zone as compared to the intact side. This involved both late negative and positive slow wave components of the cord dorsum response. The effect on the positive wave was especially pronounced when stimuli preferential for small diameter fiber activation were used. Response modalities, receptive fields and latencies for 248 isolated cells were determined using natural and electrical stimuli. The spontaneously firing of cells on the avulsed side, located at a mean depth of 1600 microns in the mid to lateral dorsal horn of the L6 or L7 could be influenced from the ipsilateral flank (L3-L4) and/or the contralateral flank and limb. This was often an inhibition. Spontaneous spike interval and post-stimulus histograms were made. Chronically avulsed cells often fired very regularly with a mean interval of 104.4 msec. The mean interval for all observed spontaneously active cells (36) in intact and control dorsal horns was 586 msec. Cells influenced from superficial receptive fields usually had irregular firing patterns, while cells having non-noxious deep receptive fields typically fired more regularly. The mean depth for cutaneous activated neurons in the mid to lateral intact dorsal horn was 1570 microns while that for deep activated cells was 2000 microns. To date no studied cell on the denervated side has been antidromically activated from the thalamus. Naloxone reversible decrease in spontaneous activity after 2.5 mg/kg Morphine has been observed in denervated cells. By immunohistochemistry, the neuropeptides met-enkephalin (ME), somatostatin (SS) and substance P (SP) have been studied at the level of injury. SP was substantially decreased through four weeks in avulsed preparations, but returned somewhat by 12 weeks. ME appeared essentially unchanged in the upper lamina of the avulsed preparation through 4 weeks, decreased by 6 weeks and remained so through 12 weeks. SS was also unchanged at 4 weeks, decreased at 6 weeks, but was returning somewhat at 12 weeks. Results from rhizotomized cords have indicated a different imbalance.

25.14 SHORT TERM CHANGES OF DORSAL HORN CELL RECEPTIVE FIELDS FOLLOWING DORSAL RHIZOTOMY IN CAT. C. J. Hodge, Jr., A. V. Apkarian and R. T. Stevens. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

Changes of the patterns of response and receptive fields of dorsal horn cells were examined in chloralose anesthetized cats following dorsal rhizotomy. Dorsal roots L2 through L6 were cut unilaterally in cats. Single unit recordings were then made in superficial dorsal horn (i.e. not deeper than 2 mm). Segments L7 through L4 were examined bilaterally with a series of regularly spaced electrode penetrations. Records from 211 cells form the basis of this report. In the immediate post-rhizotomy period the receptive field organization of all the cord segments was the same as is seen in animals with intact dorsal roots with the exception that there were fewer cells found per penetration ipsilateral to the rhizotomies in segments 4 through 6.

In the period beginning 6 - 10 hours after rhizotomy there was a striking change in the receptive field characteristics of some dorsal horn units both in the denervated portion of the spinal cord and in the non-denervated areas of the cord. A large number of cells with either bilateral or only contralateral receptive fields were found. In the immediate period only 4 of 95 cells had bilateral or contralateral receptive fields while in the later period (i.e. 6 or more hours after rhizotomy) 39 of 116 cells were found to have contralateral input. These cells had large receptive fields and almost exclusively responded to noxious stimuli. They were found proportionately more frequently in the lateral dorsal horn and at a greater depth than cells with purely ipsilateral receptive fields. These changes were as prominent in the non-denervated segments of the cord (L7) as in the denervated segments. With this exception, the somatotopic organization of the dorsal horn remained unchanged. The findings suggest that there exists extensive bilateral input into the dorsal horn, and that much of the input from the dermatomes of the contralateral side of the animal are suppressed. The mechanism by which rhizotomy unmasks this contralateral input is not yet clear.

25.15 PRIMARY NEURONS MAINTAIN THEIR CENTRAL AXONAL ARBORS IN THE DORSAL HORN FOLLOWING PERIPHERAL NERVE INJURY. T. Sugimoto* and S. Gobel. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

This study was designed to examine whether the cell bodies and central processes of primary neurons in adult cats survive up to 3 months after their peripheral branches are cut and prevented from regenerating. One superficial radial nerve was transected in the mid-forearm and capped with a polyethylene tube sealed at one end (experimental side) while the superficial radial nerve on the other side (control side) was left intact. Either 1 month or 3 months later, both nerves were transected again in the mid-forearm. Both nerves had HRP applied to their proximal stumps and were capped. Three days later the C_6-C_8 dorsal root ganglia and corresponding spinal cord segments were processed for HRP reaction product using the TMB method.

At each survival time, many cell bodies were labeled in C_6-C_8 ganglia on the experimental side as well as on the control side. The frequency distribution of labeled cell diameters (average of long and short diameters) was similiar in experimental and control sides at both survival times. The diameter of labeled cells ranged from about 10 μm to about 100 μm and about 60% of these cells fell between 20 and 40 μm in diameter. The distribution of HRP reaction product in the central axonal processes in the spinal cord was similar on the experimental and control sides at both survival times. The HRP-labeled central axonal projection extended throughout the full depth of the dorsal horn from the rostral part of C_6 through the caudal part of C_8. Most of the projection lay medial to an imaginary line extending between Lissauer's tract and the ventromedial part of lamina VI and did not fully extend to the medial border of the dorsal horn. The projection was heaviest in the head of the dorsal horn across laminae I-IV of the C_7 segment. HRP-labeled primary axonal endings throughout laminae I-IV on the experimental side of the C_7 dorsal horn were examined with EM at one month after nerve transection. All HRP-labeled primary axonal endings were apparently healthy. They were packed with synaptic vesicles and normal mitochondria and were not blackened. These primary axonal endings most commonly were presynaptic to dendritic spines and sometimes postsynaptic to other synaptic vesicle containing processes.

In conclusion, primary neurons of all sizes survive transection and capping of their peripheral processes in the superficial radial nerve for 3 months in adult cats. These injured primary neurons are capable of transganglionic transport of HRP into their central axonal arbors which maintain their normal topographic position across laminae I-VI of the C_6-C_8 dorsal horn. They remain morphologically intact and maintain their synaptic vesicles and some of their synaptic connections.

25.16 CONVERGENCE ONTO INTERNEURONS MEDIATING PAD FROM GROUP I MUSCLE AFFERENTS. EE.Brink, E.Jankoswka*, B.Skoog*, Dept. of Physiol., Univ. of Göteborg, Göteborg, Sweden.

Group I muscle afferents produce primary afferent depolarization (PAD) in different classes of afferents, depending on whether the source afferents are 1a or 1b, and whether from flexors or extensors.Notably, 1a afferents depolarize only other 1a afferents, which may also be depolarized only by 1b afferents (cf Schmidt, Ergebn.Physiol.63:20,1971). The method of spatial facilitation has now been used to determine whether the underlying paths are shared or distinct.

Dorsal root potentials (DRPs) were recorded from caudal most L_6 dorsal root filaments in chloralose-anesthetized cats. 1a afferents from TA-EDL or from GS-PL were selectively stimulated by brief small ($\lesssim$35um) muscle stretches below threshold for 1b afferent activation. 1b afferents were selectively activated by weak electrical stimulation of muscle nerves after 1a afferent thresholds had been increased by prolonged muscle vibration (Coppin et.al, J.Physiol.210:18,1970). 1a afferents of Q and PBSt were selectively activated by weak electrical stimuli ($\lesssim$1.3T). When facilitation occurred the response to simultaneous stimulation of two sets of afferents was greater than the sum of the responses to each alone.

Results showed that shared interneurons are involved in PAD pathways from different 1a afferents, whether from flexors or extensors, and in paths from flexor 1b and flexor or extensor 1a afferents. The convergence undoubtably occurred in paths to 1a afferents. In contrast, there was no convergence in paths from extensor 1b and any source of 1a afferents, although convergence occurred in paths from different extensor 1b afferents. Thus, extensor 1b afferents must use separate paths to depolarize 1a afferents. Latencies of the DRPs depend on co-excitation ranged from 2 to 7ms, indicating that convergence occurred in the shortest (trisynaptic) as well as longer paths. Finally, stimulation of cutaneous, joint or interosseous afferents at $\gtrsim$1.5T depressed 1a-induced DRPs: weaker stimuli had no effects.

The differing patterns of convergence aid identification amongst subgroups of PAD interneurons as well as their distinction from interneurons located in the same region of the spinal cord and involved in other Group I effects, as the 1b(and 1a-like-1b) postsynaptic effects in the motoneurons. Further, the patterns of convergence of segemtal inputs indicate differing control of pre- vs post- synaptic inhibition in spinal motoneurons.

25.17 LUMBOSACRAL LAMINA I CELLS PROJECTING TO MEDIAL AND/OR LATERAL THALAMUS IN THE CAT. A. D. Craig and K.-D. Kniffki*. Physiologisches Institut, Univ. Kiel, D-2300 Kiel, F. R. G.

Relatively little is known about the physiological characteristics of spinothalamic lamina I (STT-lamI) cells, especially those that project to medial thalamus, where a major STT-lamI termination occurs in the n. submedius (Craig and Burton, J. Neurophysiol., 45:443, 1981). The purpose of the present study is to examine the responses of feline STT-lamI cells that project to medial and/or lateral thalamus to inputs from skin and muscle.

Recordings are made from cells in the vicinity of lamina I with tungsten microelectrodes in chloralose-anesthetized cats. Recording sites are verified by small lesions. A linear array of ten bipolar electrodes, inserted at the caudal end of the somatosensory thalamus (AP 7.0) as judged by recording coordinates, is used to activate cells antidromically (standard criteria). Cells are characterized by electrical stimulation of sciatic, tibial, gastrocnemius-soleus (GS), and sural nerves, stimulation of GS group III and IV afferents by close intra-arterial injection of KCl and bradykinin, and natural stimulation of skin and muscle.

To date, 71 STT-lamI cells have been recorded in the lumbosacral spinal cord (L5 - S1) with a mean antidromic latency of 82 ms (range = 25 - 250 ms). Of these, 54% projected only to medial thalamus, 20% only to lateral thalamus, and the remainder to both or to mid-thalamus. A sample of 21 STT-lamI cells has been fully characterized by their response properties to peripheral stimulation and their thalamic projection. Almost all (19) of these cells were activated specifically by noxious stimulation; 14 responded to pinch and/or noxious radiant heat applied to the skin, 3 received convergent noxious skin and deep input, and 2 responded only to noxious deep input. These 19 nociceptive-specific cells project to thalamus as follows: 10 medial, 2 lateral, and 7 medial + lateral. Similarly, almost all the cells (17/19) with a projection to medial thalamus were nociceptive specific. Of the remaining 2 cells, 1 responded only to cooling the skin, and the other was a cutaneous wide dynamic range cell.

These results indicate that lumbosacral STT-lamI cells in the cat are carrying ascending information about noxious stimulation of both skin and muscle. Furthermore, the majority of lumbosacral STT-lamI cells appears to project to medial thalamus (perhaps to the n. submedius) and to be nociceptive specific.

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25.18 INTRACELLULAR STAINING OF MULTIRECEPTIVE NEURONS IN CAT LAMINAE III-VII. L.A. Ritz and J.D. Greenspan. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27514.

We have investigated the structure-function relationship of multireceptive neurons in laminae III-VII of the coccygeal cord of the decerebrate cat by intracellularly staining physiologically studied neurons with horseradish peroxidase (HRP).

We studied over 100 units evidencing input from more than one cutaneous receptor type. Among these, 50 units had input from both myelinated and unmyelinated primary afferent fibers and 55 units received input from both thickly and thinly myelinated afferent fibers. A single unit's response properties were first determined by extracellular recording, and, after intracellular penetration and confirmation of response characteristics, we passed iontophoretic current to deposit HRP.

We obtained 12 examples of well stained, single neurons, including 2 with cell bodies in the dorsal nucleus proprius, 7 with cell bodies in lamina V and 3 with cell bodies in lamina VII. Eight of the 12 units had late discharges correlated with the C-wave of a doral root recording. Only one of these 8 had dendrites that reached the superficial dorsal horn. Of the 4 units with no C input, 2 had dorsal dendrites entirely contained within the nucleus proprius and 2 had dorsally passing dendrites with evidence of branching in lamina I.

Six of the 7 lamina V located cell bodies received input from both low and high threshold myelinated mechanoreceptive fibers, while the 7th unit received only high threshold mechanoreceptive input; 4 of these 7 units evidenced input form both A and C fibers, including the one nociceptive-specific unit. The cell body sizes for lamina V neurons ranged from 30 to 73 micrometers in the largest dimension. Each neuron had an extensive dendritic spread in several directions; most noteworthy was the rostrocaudal extension of 800 to 1200 micrometers. Six lamina V neurons had lateral dendrites that reached into the lateral white matter; 2 of these 6 also had medial dendrites in the gray matter over the central canal. Four lamina V neurons gave rise to axons which entered the contralateral ventral white matter; one of these also had an axon collateral with many branches deep in the ipsilateral ventral horn.

There does not appear to be a correlation between the presence or absence of C-fiber input and the dendritic extent or orientation of these multireceptive neurons. The lamina V neurons, all of which received input from cutaneous mechanical nociceptors were large and had extensive dendritic domains.

This study was supported by PHS grant NS-10321 and fellowship awards NS-06347 and NS-06835.

25.19 THE CELLS OF ORIGIN OF THE MONKEY'S DORSAL COLUMN POSTSYNAPTIC SPINOMEDULLARY (DCPS) SYSTEM. G.W. Lu*, G.J. Bennett, N. Nishikawa* and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, Maryland 20205.

The DCPS system consists of spinal neurons whose axons ascend through the dorsal columns to terminate in the dorsal column nuclei. We have previously reported the location and distribution of the cat's lumbosacral DCPS neurons (Soc. Neurosci. Abstr., 7: 611, 1981) and now report the location and distribution of lumbosacral DCPS neurons in macaque monkeys.

The dorsal columns (DC) of 1 cynomolgus and 3 rhesus monkeys were partially transected in mid or lower thoracic segments. Two monkeys also received bilateral lesions of the dorsolateral funiculi 1-3 cm caudal to their DC transections. A small piece of HRP-containing polyacrylamide gel was inserted into the DC transection, the spinal cord covered with Gelfoam, and the animals allowed to recover. They were sacrificed and perfused 24-68 h later. The retrograde label was developed with TMB.

Dorsolateral funicular lesions had no effect on the frequency or distribution of labeled neurons. Retrogradely labeled cells were concentrated in a broad band in laminae III-IV. Scattered cells were found in laminae V-VII, X, and the dorsolateral white matter. The monkey's distribution is thus different than the cat's where most DCPS cells are concentrated in a narrow band in lamina IV that sweeps down through laminae V-VI along the dorsal horn's medial border.

DCPS neurons were counted in three monkeys. The lumbosacral enlargements (L_4-S_1) contained 682-1,139 cells. Average cell densities ranged from 14.8-41.8 cells in each unilateral millimeter of the enlargement's length. These densities are nearly identical to what we found in cats. Thus in the monkey, as in the cat, DCPS neurons are as numerous as spinocervical neurons but 2-4X less numerous than spinothalamic neurons.

The retrograde label filled the cells' proximal dendritic arbors out to the 2^o and 3^o dendrites. Laminae III-V neurons had dendritic arbors that were relatively narrow mediolaterally and elongated rostrocaudally. Cells found towards the medial borders of laminae V-VII had arbors that fanned-out laterally. Lamina X cells were small and bipolar.

We conclude that, in both the cat and monkey, the DCPS projection is a major source of spinal input to the brain.

25.20 INDIVIDUAL THORACIC SPINAL NEURONS WITH MULTIPLE TERMINATION SITES IN THE RETICULAR FORMATION AND THALAMUS AND THEIR RESPONSES TO INTRACARDIAC INJECTIONS OF BRADYKININ IN THE CAT. R. Neal Weber, Robert W. Blair and Robert D. Foreman, Dept. of Physiol. and Biophys. Univ. of Oklahoma HSC, Okla. City, OK 73190.

Previous studies from our laboratory have shown that noxious stimulation of the heart by bradykinin can alter the activity of spinoreticular neurons in the cat and spinothalamic neurons in the monkey. In these studies the antidromic stimulating electrodes were located bilaterally in the medullary reticular formation (RF) or in the ventral posterior lateral region of the contralateral thalamus of 39 alpha-chloralose anesthetized cats. We recorded from 71 antidromically activated cells found in the T_2-T_4 segments of the left thoracic gray matter. Six different types of rostrally projecting neurons were identified according to the site or sites from which they could be activated: those activated from the 1) contralateral RF (CRF) only, 34 of 71 cells (48%); 2) ipsilateral RF (IRF) only, 12 cells (17%); 3) thalamus only, 9 cells (13%); 4) both CRF and IRF, 8 cells (11%); 5) both CRF and thalamus, 4 cells (6%); and 6) CRF, IRF and thalamus 4 cells (6%). The cell bodies of these neurons were located in laminae I, IV, V, VI, VII, and VIII; the majority were found in V and laminae VII, but no obvious relationship was found between the sites of activation and laminar location. Each of the cells exhibited convergence of sensory input from somatic receptive fields and from sympathetic afferent fibers. Cell activity of 44 spinoreticular and spinothalamic cells was tested for changes following injection of bradykinin (1-2 ug/kg) into the left atrium. Twenty five cells were excited, one cell was inhibited and 32 cells were unaffected following the injection. Average latency from the time of injection of bradykinin to onset of the response was 13s and average response duration was 48s. Average response increased from a control rate of 2 Hz to a peak rate of 16 Hz. Three of 15 cells tested responded to control injections into the descending aorta but with longer latency and less peak activity. Cells were significantly more likely to respond with a greater discharge rate to intracardiac bradykinin injections if they received afferent input from both Aδ and C-fibers than if the input was from Aδ fibers alone. A marked tachyphylaxis to repeated injections of bradykinin developed for 50% of the cells which responded to an initial dose of BK. The six different types of rostrally projecting neurons responded similarly to intracardiac injections of bradykinin. To conclude, since individual spinal neurons projecting to both the reticular formation and thalamus respond to intracardiac BK, both regions receive input when the heart is injured. Supported by National Heart, Lung and Blood Institute Grants HL22732, HL07430 and HL00557.

26.1 DYNORPHIN IMMUNOREACTIVITY IN RAT AND GUINEA PIG NERVOUS SYSTEMS, S.R. Vincent, T. Hökfelt*, I. Christensson*, L. Terenius*, C.-J. Dalsgaard* and M. Schultzberg*. Dept. of Histology and Anatomy, Karolinska Institutet, Stockholm & Dept. of Pharmacology, Uppsala University, Uppsala, Sweden.

Antibodies raised against the endogenous opioid peptide dynorphin-(1-17) (DYN) and specific for the C-terminal region of the peptide were used to examine the distribution of DYN immunoreactivity in the nervous system. The antiserum used (84D) did not cross-react with leucine enkephalin in either radioimmunoassay or immunohistochemistry.

Within the autonomic nervous system, intense networks of DYN-positive nerve fibers were observed in the prevertebral ganglia (inferior mesenteric, coeliac-superior mesenteric), while the paravertebral ganglia (superior cervical, stellate) contained few DYN fibers. DYN-positive fibers were also seen in the myenteric plexi of the duodenum and the proximal and distal colon. SIF cells containing intense DYN immunoreactivity were observed in the superior cervical ganglion and inferior mesenteric ganglion of the guinea pig. The adrenal medulla contained a few DYN-positive nerve fibers, however, chromaffin cells were not DYN-immunoreactive, although they displayed intense enkephalin staining on adjacent sections.

In the spinal cord and medulla DYN cell bodies and fibers were concentrated within the substantia gelatinosa. No DYN-immunoreactivity was observed in the spinal ganglia. DYN fibers and cell bodies were also observed in the nucleus of the solitary tract and in the parabrachial nucleus. The substantia nigra contained an intense network of DYN-positive fibers in the pars reticulata. This network of fibers was very similar to that previously noted for both GABA and substance P in the nigra and showed a similar decrease in the nigra following striatal lesions with ibotenic acid, suggesting the existence of a major striato-nigral DYN pathway. A small group of DYN-postive cells was present just dorso-lateral to the substantia nigra. In the hypothalamus, major DYN cell groups were observed in the ventral premamillary nucleus, the supraoptic and paraventricular nuclei, and especially in the region of the medial forebrain bundle just between the internal capsule and the fornix. DYN cells were also observed in the bed nucleus of the stria terminalis, the central nucleus of the amygdala, and the granule cell layer of the dentate gyrus. The stria terminalis contained many positive nerve fibers. The neocortex contained only single DYN fibers, while the cerebellum did not display DYN-immunoreactivity.

The results provide evidence for an extensive DYN system throughout all levels of the nervous system, distinct from the enkephalin or other opioid systems previously described.

26.2 ENKEPHALINERGIC PATHWAYS IN THE PRENATAL RAT: ORGANIZATION IN SITU AND TRANSPLANTATION IN OCULO. M. Palmer, B. Hoffer*, H. Björklund*†, Å. Seiger*† and L. Olson*†. Univ. Colorado Health Sciences Center, Denver, Colo., and †Karolinska Institute, Stockholm, Sweden.

Immunohistochemical fluorscence techniques were used to determine the presence of endogenous enkephalin-like immunoreactivity (ELI) in the developing central nervous system of the prenatal rat. ELI was first observed in beaded fibers along the midline of ventral pons and cervical spinal cord by postconception day 15. Fluorescence could not be observed in the brains of 14-day-old fetuses, although the adrenal medulla did show ELI at this stage. ELI-positive cell bodies at postconception day 18 were found in most areas in which they have previously been described in adult rats. Many of the cells observed at postconception day 18 were, however, not seen in noncolchicine-treated full-term fetuses with the techniques used here. ELI-positive fibers and terminal fields began to approach adult distributions at postconception day 20-21. Several ELI-positive axon pathways which have not been previously reported in adult or developing brain, for example, pathways in medial neocortex, in fasciculus retroflexus, in tractus mammillothalamicus, between ventral hypothalamus and globus pallidus, and between ventromedial pons and the locus coeruleus area, were observed. Areas of fetal brain and spinal cord tissue, known to contain ELI-positive neurons, were then homologously grafted into the anterior chamber of the eye of adult recipients. These neurons survived well in oculo and elaborated a dense plexus of ELI-positive fibers in the graft. Taken together, these data suggest enkephalinergic pathways develop long before birth in the rat, at an age early enough to permit survival after transplantation to the anterior chamber. (Supported by the Swedish MRC Grants # 14X-03185 and 14P-5867, and NIDA Grant # DA-02702.)

26.3 STUDIES ON THE RELATIVE DISTRIBUTION OF α-NEO-ENDORPHIN/DYNORPHIN AND PROENKEPHALIN NEURONAL SYSTEMS AND THEIR PEPTIDE PRODUCTS. Eckard Weber*, Kevin Roth*, Christopher J. Evans* and Jack D. Barchas. (SPON: W. C. Dement). Nancy Pritzker Laboratory of Behavioral Neurochemistry, Stanford Medical Center, Stanford, CA 94305.

Previous studies have demonstrated that α-Neo-endorphin and dynorphin(1-17) immunoreactivity are colocalized within the same neurons of the rat brain (1,2). Subsequent biochemical analysis of α-Neo-endorphin and dynorphin related peptides in 10 brain regions has shown that the principal dynorphin-related peptide is dynorphin(1-8), which is present in up to 10-fold higher concentrations in brain than dynorphin(1-17) (3). Dynorphin(1-8) but not dynorphin(1-17) is present in equimolar concentrations with α-Neo-endorphin in all brain regions (3). These results imply that dynorphin(1-8) and α-Neo-endorphin are biosynthetically closely related and that dynorphin(1-17) may be a precursor in the formation of dynorphin(1-8). Both our biochemical and immunohistochemical studies suggested that the α-Neo-endorphin/dynorphin neuronal system shows a significant overlap with enkephalin pentapeptide neurons. Most of the enkephalins seem to be derived from the recently characterized pro-enkephalin molecule, which does not contain dynorphin(1-8), dynorphin(1-17) nor α-Neo-endorphin. Therefore, a very important question is whether there are neurons which contain pro-enkephalin products as well as dynorphin and α-Neo-endorphin or whether the α-Neo-endorphin/dynorphin neuronal system is strictly separate from the pro-enkephalin neuronal system. To address this question, we have prepared antibodies specific for pro-enkephalin products (met-enkephalin-Arg^6-Phe^7 and met-enkephalin-Arg^6-Gly^7-Leu^8) and have compared the distribution of these peptides with the distribution of peptides specific to the α-Neo-endorphin/dynorphin system [dynorphin(1-8), -(9-17), α-Neo-endorphin]. The results as of this writing suggest that there is a partial overlap of pro-enkephalin neurons and α-Neo-endorphin/dynorphin neurons.

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26.4 CIRCADIAN VARIATION OF BETA ENDORPHIN-LIKE IMMUNOREACTIVITY IN THE ADRENAL GLAND AND SPINAL CORD. K.D. Gipson*, A.D. Okonmoh*, and C.A. Walker, School of Pharmacy, Florida A&M Univ., Tallahassee, FL 32307

A circadian rhythm for beta endorphin-like immunoreactivity in the adrenal gland and spinal cord has been established. Male Sprague-Dawley (200-250g) rats adapted to a 12h light-12h dark programmed illumination cycle and controlled temperature (23 + 1°C) for a minimum of three weeks were used in this study. Animals were sacrificed by decapitation at 4 hr. intervals during the 24 hr. period. The adrenal gland and cervical region of the spinal cord were dissected and extracted with 0.1N HC1. Beta endorphin-like immunoreactivity content was measured by radioimmunoassay. This method employed addition of delayed tracer to improve sensitivity. Phase separation was accomplished by addition of goat anti-rabbit serum in the presence of carrier rabbit serum which is titered to assure mid-zone precipitation. Results of this study show that there is a significant difference between peak and trough levels in both tissues with the peak occurring in the dark phase and the trough during the light phase. These results are in agreement with previous studies conducted in our lab which demonstrated circadian changes in beta-endorphin levels in the hypothalamus and periaqueductal gray-rostal pons regions of the brain. These findings also agree with studies which have demonstrated diurnal changes in responsivity to pain stimuli and morphine induced analgesia. (Supported in part by NASA).

26.5 DEMONSTRATION OF CONTACTS BETWEEN OPIOCORTIN NEURONS IN THE ARCUATE NUCLEUS OF THE RAT HYPOTHALAMUS. G. Pelletier and Y.Y. Chen*. MRC Group in Molecular Endocrinology, CHUL, Québec, Canada G1V4G2.

It has been recently established by immunocytochemistry that peptides derived from proopiocortin, namely ACTH, α-MSH and endorphins, are produced by the same neurons in the rat arcuate nucleus (Watson et al., Nature, 275:66, 1978; Guy et al., Brain Res. 199:135, 1980). These neurons, named opiocortin neurons, are projecting into several brain areas, including several hypothalamic nuclei, septum, thalamus and midbrain. In order to identify target neurons for the opiocortin system, we first investigated the possibility that opiocortin neurons can contact themselves. For this purpose, we extensively studied the localization of immunoreactive ACTH and endorphins in the rat arcuate nucleus. Immunostaining was performed on Vibratome sections using a pre-embedding staining technique described by Pickell et al. (PNAS, 72:658, 1975). In 1.5 μm thick plastic sections, very close contacts between immunostained endings and cell bodies or dendrites were routinely observed. Using serial sections, it was frequently observed that the same positive axon could have contacts with more than one positive cell body. At the ultrastructural level, immunostained neuronal cell bodies, dendrites and endings were routinely detected. All the positive cell bodies and endings contained dense core vesicles of a diameter ranging between 70-120 nm. Twenty-five positive endings making contact with immunostained cell bodies or dendrites were observed. The contacting endings were frequently seen in an area of the cell body or dendrite presenting an invagination in its plasma membrane. No synaptic densities could be observed in the zone of contact between a positive ending and the contacted cell body or dendrite. These observations clearly indicate that neurons producing the same peptides can have contacts between themselves. They provide morphological evidence for autoregulation of neuropeptide systems.

26.6 DIFFERENTIAL IMMUNOCYTOCHEMISTRY OF LEU-ENKEPHALIN AND DYNORPHIN IN THE RAT SPINAL CORD. J.E. Kelsey*, H. Khachaturian, and S.J. Watson (SPON: D. Goodman). Mental Health Res. Inst., Univ. of Mich., Ann Arbor, MI 48109.

Leu-enkephalin has been shown to be distributed throughout the spinal cord gray matter with highest concentrations occurring in the dorsal horn. Dynorphin, which contains Leu-enkephalin at its NH_2 terminus has also been localized to the spinal cord. Due to the possibility of cross-reactivity between antisera generated against these peptides, we have employed antisera directed against the full sequence of each peptide, as well as sequences unique to the dynorphin molecule (i.e. Dynorphin-(7-17); produced in collaboration with D.H. Coy, Tulane Univ.) and to the non-enkephalin portion of BAM-22P (a peptide fragment from the adrenal enkephalin precursor). This has enabled us to differentiate between dynorphin and enkephalin systems. Adult male Sprague-Dawley rats were pretreated with icv colchicine. Animals were perfused with formaldehyde and the spinal cords were processed for PAP immunocytochemistry. Cross-blocking studies demonstrated dynorphin-(1-17) and -(7-17) immunoreactivities to be separable from Leu-enkephalin and BAM-22P immunoreactivities. Comparison of Leu-enkephalin and BAM-22P to dynorphin-(1-17) and -(7-17) immunoreactivities in the spinal cord demonstrated many similarities. The highest density of Leu-enkephalin immunoreactive fibers and terminals occurred in the marginal zone of the dorsal horn and as a group of fibers in the white matter immediately lateral to the marginal zone. Scattered varicosities were also noted in the deeper laminae of the dorsal horn, around the central canal, and to a lesser extent in the ventral horn. Occassional small immunoreactive perikarya were also noted in the deeper laminae of the dorsal horn. Immunoreactive dynorphin fibers and terminals appeared to be present in all of the above areas, but to a lesser extent than Leu-enkephalin immunoreactivity. We were unable to detect dynorphin positive perikarya in the dorsal horn. The distribution of these peptides in the dorsal horn of the spinal cord is consistent with their role in nociception. The similar localization of Leu-enkephalin and dynorphin immunoreactivities in the spinal cord raises the question of their differential post-synaptic effects, and the possibility of enzymatic conversion of some dynorphin to Leu-enkephalin.

26.7 VISUALIZATION OF OPIATE RECEPTORS IN RELATION TO OPIOID PEPTIDE NEURONAL SYSTEMS IN BRAIN AND SPINAL CORD. M.E. Lewis*, H. Khachaturian and S.J. Watson (SPON: E.F. Domino). Mental Health Res. Inst., Univ. of Mich., Ann Arbor, MI 48109.

Immunocytochemical studies of opioid peptide localization and autoradiographic studies of opiate receptor distribution have previously been carried out in different animals, precluding a direct correlation of their loci in brain. To carry out correlative autoradiographic-immunocytochemical studies, we perfused rats with 0.1 M phosphate-buffered 4% formaldehyde (pH 7.4) and obtained 20μm-thick sections of their brains with a cryostat. Opiate receptors$_3$ (μsubtype) were labelled selectively by incubation with 1 nM [^{3}H]naloxone (in 0.05 M Tris HCl, pH 7.4, containing 100 mM NaCl) at 4°C for 1 hr, followed by 5 successive 20 sec rinses in 0.1 M phosphate-buffered saline at 4° C to remove most nonspecific binding. Receptor specificity studies, carried out by liquid scintillation counting of incubated striatal sections, indicated a partial loss of specific [^{3}H]naloxone binding sites due to perfusion, with no apparent change in the pharmacological properties of the remaining sites as indicated by competition binding curves. For the anatomical studies, [^{3}H]naloxone-labelled brain sections from colchicine-treated, perfused rats were fixed with paraformaldehyde vapors at 80°C for 2 hr and processed for liquid emulsion autoradiography (Herkenham and Pert method) and alternate sections were processed for immunocytochemistry using affinity-purified Leu-enkephalin antisera in the PAP procedure (see Khachaturian et al., this meeting). The radiolabelled sections could not be used for immunocytochemistry since the vapor fixation destroyed all enkephalin immunoreactivity. To avoid the necessity of the vapor fixation step, [^{3}H]naloxone-labelled sections were apposed to LKB Ultrofilm or dry, emulsion-coated coverslips (Young and Kuhar method) and later processed for immunocytochemistry. Excellent correlations between the loci of [^{3}H]naloxone binding sites and enkephalin immunoreactivity were observed in a number of areas, including the dorsal horn of the spinal cord, spinal trigeminal nuc., nuc. tractus solitarius, parabrachial nuc., area postrema, interpeduncular nuc., substantia nigra, ventral tegmental nuc., periaqueductal gray, hippocampus, nucleus accumbens and some thalamic, cerebral cortical and olfactory bulb areas. The procedures described here permit, for the first time, direct anatomical correlations between the localization of opiate receptors and opioid peptides. These methods are currently being used for correlative studies visualizing μ, δ and κ opiate receptors in relation to β-endorphin, enkephalin and dynorphin neuronal systems in rat and monkey central nervous system.

26.8 COMPARATIVE BRAIN DISTRIBUTION OF ENKEPHALIN AND AN ENKEPHALIN PRECURSOR FRAGMENT. H. Khachaturian, M.E. Lewis*, V. Hollt[1] and S.J. Watson, Mental Health Research Inst., Univ. of Mich., Ann Arbor, MI 48109; [1]Max Planck Institute for Psychiatry, Munich, West Germany.

The comparative distribution of Leu-enkephalin and an enkephalin precursor fragment, bovine adrenal medullary docosapeptide (BAM-22P), was studied in the rat brain using immunocytochemical procedures. The full amino acid sequence of BAM-22P is as follows: Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-Gly-Arg-Pro-Glu-Trp-Trp-Met-Asp-Tyr-Gln-Lys-Arg-Tyr-Gly. In this report, we provide evidence for the presence of the enkephalin precursor processing system in the brain. Rats were treated intracerebroventricularly with colchicine (300-400ug) to enhance the visualization of neuronal perikarya. The peroxidase-anti-peroxidase technique of immunocytochemistry was carried out on formaldehyde-fixed, frozen sections, using antisera generated against each peptide. Careful cross-blocking paradigms confirmed the differential antigenicity of each serum.

The use of high doses of colchicine permitted the visualization of widespread enkephalin-containing systems in the brain, some of which have not yet been fully described. Leu-enkephalin immunoreactive perikarya or fibers and terminals were noted in the olfactory bulb, many neocortical and paleocortical regions of the cerebrum, amygdala, hippocampus, basal ganglia, septum, thalamus, hypothalamus, mesencephalic periaqueductal gray, the colliculi, many brain stem reticular formation sites, including some cranial nerve nuclei, and almost all monoamine-containing regions. In most of the above areas, we have also noted at least some BAM-22P immunoreactivity. Furthermore, through serial-section analysis, it has been possible to confirm the close anatomical association between BAM-22P and Leu-enkephalin immunoreactivities; i.e., these immunoreactivies were localized to perikarya and processes in the same region. Studies are in progress to evaluate the possibility of co-localization of these peptides within the same neurons. Nevertheless, the results suggest that brain enkephalin-positive neurons may possess mechanisms for enkephalin biosynthesis similar to those found in adrenal medullary cells.

26.9 DYNORPHIN NEURONAL SYSTEMS: DISTRIBUTION IN BRAIN AND RELATIONSHIP TO OTHER OPIOID PEPTIDES. S.J. Watson, H. Akil, and H. Khachaturian. Mental Health Res. Inst., Univ. of Mich., Ann Arbor, MI 48109.

The opioid peptide dynorphin was extracted and sequenced from nervous tissue. To date this peptide has been biochemically detected throughout the neuroaxis and anatomically localized in a few loci. We previously reported it's visualization in posterior pituitary, and the magnocellular neurosecretory nuclei of hypothalamus in the vasopressin producing cells in normal rats and the magnocellular nuclei of homozygous Brattleboro rats. Because the structure of dynorphin contains leucine-enkephalin we have been concerned that antisera against leucine-enkephalin and dynorphin might cross-react under immunohistochemical conditions, thereby confusing the anatomy of the enkephalin and dynorphin neuronal systems. We have carried out a series of blocking studies using the several opioid peptides across a variety of antisera and can reliably separate the two systems from each other (and from the beta-endorphin system as well).

With the publication of the mRNA structure coding for adrenal proenkephalin it has been possible to eliminate this precursor as a source for dynorphin thereby strongly suggesting a unique dynorphin biosynthetic route. As reported in a separate poster at this meeting (Khachaturian et al) we have used enkephalin and an enkephalin precursor peptide (BAM-22P) antisera to identify enkephalin cells in brain. Dynorphin cells were stained with antisera against dynorphin-(1-13), (1-17) and (7-17).

These two sets of sera (leu-enk, BAM-22P vs. dynorphin-(1-13), (1-17), (7-17)) have allowed consistent separation of these peptide systems in several brain loci.

The dynorphin system in brain is very wide spread including the olfactory bulb, n. accumbens, lateral septal nucleus, globus pallidus, the hypothalamic magnocellular nuclei, the arcuate nucleus, central nucleus of amygdala, several cortical layers, periaqueductal grey, n. parabrachialis, reticular formation, n. gracilus, raphe nuclei, nucleus tractus solitarius, and spinal cord. It composes the third major opioid peptide system in brain.

26.10 IMMUNOHISTOCHEMICAL LOCALIZATION OF ENKEPHALIN-, AND ACTH-RELATED SUBSTANCES IN THE ADENOHYPOPHYSIS OF THE LAMPREY. R. M. Dores, T. E. Finger and M. R. Gold. Depts. of Physiology and Anatomy, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

In the mammalian adenohypophysis, ACTH- and β-endorphin-related polypeptides are synthesized on a common precursor molecule (pro-ACTH/endorphin). Consequently, antisera directed against these peptides react with the same populations of cells. However, the enkephalins, which are not synthesized from β-endorphin, are found only in the neurohypophysis. In this study the distributions of immunoreactive forms of these peptides were assessed in the lamprey. Brains with pituitaries attached were dissected from brook lampreys, Lampetra lamottenii, and fixed with periodate-lysine-paraformaldehyde. The tissue was processed for immunocytochemistry by the indirect peroxidase-anti-peroxidase procedure utilizing a metal intensification of the DAB reaction. The primary antisera were raised in rabbits and were specific for the following: the middle region of ACTH, the NH_2-terminal of ACTH, β-endorphin, γLPH, 16K fragment, met-enkephalin and leu-enkephalin. These antisera were used at dilutions of 1:100 to 1:2000. Blocking experiments involved preabsorption of the antisera with 10 μM of the appropriate peptide. The lamprey adenohypophysis is divisible into three regions: the pro- and meso-adenohypophysis (purported homologs of the pars distalis), and the meta-adenohypophysis (purported homolog of the pars intermedia). In the pro-adenohypophysis a cluster of cells exhibited immunoreactivity with the middle ACTH antiserum and both enkephalin antisera, but did not react with any other antisera. The ACTH reactivity was blocked with ACTH(1-24), and the enkephalin reactivities were blocked with enkephalin but not with β-endorphin(1-31). In the meso-adenohypophysis a small population of cells exhibited immunoreactivity only with the γLPH antiserum. This reactivity was blocked with βLPH but not with β-endorphin(1-31). In the meta-adenohypophysis all cells reacted with the NH_2-terminal ACTH antiserum and both enkephalin antisera; however, these cells did not crossreact with any of the other antisera. The NH_2-terminal ACTH reactivity was blocked with αMSH, and the enkephalin reactivities were blocked with enkephalin but not with β-endorphin(1-31). In the neurohypophysis met- and leu-enkephalin immunoreactive fibers were detected. The absence of βLPH/β-endorphin immunoreactivity coincident with ACTH immunoreactivity, and the immunohistochemical detection of enkephalin in the adenohypophysis are unique to the lamprey. These observations suggest that there have been marked changes during evolution of the ACTH/βLPH family of pituitary hormones.
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26.11 QUANTITATION AND LIGHT AND ELECTRON MICROSCOPIC STUDIES OF IMMUNOREACTIVE α-NEO-ENDORPHIN/DYNORPHIN RELATED PEPTIDES IN THE RAT SUBSTANTIA NIGRA. Kevin A. Roth*, Eckard Weber* and Jack D. Barchas. (SPON: D. L. Wong) Nancy Pritzker Laboratory of Behavioral Neurochemistry, Stanford Medical Center, Stanford, CA 94305.

In light microscopic studies we have observed an intense granular, terminal-like immunofluorescence in the substantia nigra for immunoreactive dynorphin(1-17) and α-Neo-endorphin (1). Dynorphin(1-8), an aminoterminal fragment of dynorphin (1-17) which is present in rat brain in much higher concentrations than dynorphin(1-17) (2), produces an identical pattern of immunostaining in substantia nigra. No immunoreactive α-Neo-endorphin/dynorphin cell bodies were visualized in the substantia nigra light microscopically. To further these studies, we have examined immunoreactive α-Neo-endorphin, dynorphin(1-8) and dynorphin(1-17) in the substantia nigra electron microscopically by a peroxidase anti-peroxidase pre-embedding staining method.

In preliminary electron microscopic studies, we have failed to observe cell bodies immunoreactive for α-Neo-endorphin/dynorphin in the substantia nigra. Specific α-Neo-endorphin/dynorphin immunoreactivity was seen predominantely in unmyelinated axons, occasional immunoreactive myelinated axons were also seen. More detailed studies are currently in progress.

We have also performed radioimmunoassays on dissected rat substantia nigras to provide quantitative data on the concentrations of α-Neo-endorphin, dynorphin(1-8), and dynorphin(1-17) in this region. This data will also be presented.

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2. E. Weber, C. J. Evans, J. D. Barchas. Opioid peptide dynorphin: Predominance of the amino-terminal octapeptide fragment in rat brain regions. Nature, (1982) in press.

26.12 IMMUNOCYTOCHEMICAL LOCALIZATION OF DYNORPHIN IMMUNOREACTIVITY IN RAT CNS. Jacqueline F. McGinty, Lars Terenius*†, and Floyd E. Bloom. The Salk Institute, La Jolla, CA and †Uppsala University, Uppsala, Sweden.

Antisera raised against enkephalin have revealed a widespread pattern of "enkephalinergic"-like immunoreactivity (ir) in the CNS. Several larger molecular weight peptides containing the sequence of either methionine-enkephalin or leucine-enkephalin have been isolated and sequenced. With an antiserum to dynorphin 1-17, an opioid peptide which contains the pentapeptide sequence of leucine-enkephalin at its N-terminus, we have been able to distinguish a specific subdivision of the enkephalinergic network in the rat CNS. Most densely stained fibers are the hippocampal mossy fibers, substantia nigra zona reticulata, and the internal zone of the median eminence. Moderately stained are fibers found within the central amygdaloid nucleus, lateral septum, bed nucleus of stria terminalis (BNST), medial preoptic ara, nucleus accumbens, caudate-putamen, hypothalamic arcuate nucleus, entopeduncular nucleus, periventricular thalamus, midbrain central gyrus, nucleus tractus solitarius, and substantia gelatinosa of trigeminal nerve and dorsal horn. Widely scattered, thick fibers are present in olfactory, limbic and neocortex. Darkly staining cells are present in the central amygdaloid nucleus without colchicine pretreatment. After colchicine treatment, clusters of cells are apparent in prefrontal and cingulate cortex, olfactory peduncle, lateral septum, BNST, hypothalamic paraventricular and supraoptic nuclei, arcuate nucleus, lateral hypothalamus, dentate granule layer, and scattered in the hippocampal CA cellular fields. No dynorphin(ir) was apparent in the lateral perforant path. Dynorphin (ir) was abolished by absorption with 1 μM dynorphin 1-17 but not 1-100 μM dynorphin 1-13, leucine-enkephalin, or α-neo-endorphin. Thus we can distinguish three types of neuronal systems which putatively contain either dynorphin (ir), enkephalin (ir) or both dynorphin/enkephalin (ir) in rat CNS.
Supported by NIDA 01785 and HL 25457.

26.13 DISTINCT DISTRIBUTION OF IMMUNOREACTIVE DYNORPHIN AND LEUCINE ENKEPHALIN IN VARIOUS POPULATIONS OF ISOLATED ADRENAL CHROMAFFIN CELLS. M. Dumont*, R. Day* and S. Lemaire. Département de Pharmacologie, Centre Hospitalier Universitaire, Sherbrooke, Québec, Canada J1H 5N4.

The distribution of immunoreactive-dynorphin (ir-Dyn) in isolated subpopulations of bovine adrenal chromaffin cells was examined and compared with that of adrenaline (A), noradrenaline (NA) and ir-leucine-enkephalin (ir-Leu-Enk). Using a stepwise bovine serum albumin (BSA) gradient, various populations of catecholamine-storing cells were differentially separated in three layers (I, II and III). Cell layer I was enriched in NA, cell layer II contained both A and NA in equal amounts and cell layer III was enriched in A. Before separation on BSA gradient, the original cell preparation contained 3.5 times more ir-Leu-Enk as ir-Dyn (5.72 and 1.63 pmoles per 10^6 cells, respectively). After separation, ir-Dyn and ir-Leu-Enk were mostly confined to cell layer I and cell layer III, respectively. The secretion of A, NA, ir-Dyn and ir-Leu-Enk by the different cell layers corresponded to their total content: the cell population I mostly secreted NA and ir-Dyn, whereas the cell population III secreted A and ir-Leu-Enk. After subcellular fractionnation of the bovine adrenal medulla, both peptides (ir-Dyn and ir-Leu-Enk) were found in the secretory granules of adrenal chromaffin cells indicating that they are costored and cosecreted with the catecholamines. The levels of [^{3}H]-naloxone and [^{3}H]-Dyn binding to each layer were also compared. [^{3}H]-naloxone and [^{3}H]-Dyn were shown to bind equally and predominantly to cell layer III. These results indicate that bovine adrenal chromaffin cells can be isolated according to their content in specific catecholamines, opioid peptides and opiate binding sites and suggest that Dyn and Leu-Enk have a distinct biosynthetic pathway.

26.14 MET- AND LEU-ENKEPHALIN IN THE GUINEA PIG HIPPOCAMPUS. Douglas W. Hoffman, Richard A. Altschuler and J. Gutierrez*. LNO, Bldg. 36, Rm. 5D32, National Institutes of Health, Bethesda, MD 20205

There are conflicting reports of the localization and molecular form of enkephalin related peptides in the hippocampus. To resolve these points high pressure liquid chromatography (HPLC) and immunochemical techniques were combined to provide unequivocal regional localization, quantitation, and molecular identification of these peptides. Immunocytochemical experiments were performed using antisera raised against enkephalin.

HPLC was performed on whole hippocampal complex from 1 month old female NIH guinea pgs. The tissue was rapidly excised, sonicated in HPLC buffer and centrifuged to pellet particulate matter. Supernatant was injected directly onto a microBondapak octadecylsilane (10 micron) column and isocratically eluted with 0.05% trifluoroacetic acid in 25% acetonitrile. Good separation of enkephalin related peptides was achieved in a short (20 min.) run time. Collected fractions were frozen, lyophilized and reconstituted in a phosphate-BSA buffer for radioimmunoassay (RIA). RIAs were performed with a commercially available met-enkephalin antiserum cross-reactive to leu-enkephalin, allowing detection and quantitation of both these peptides in one chromatographic run and immunoassay. A third fraction of immunoreactivity which was neither leu- nor met-enkephalin was also observed in 7 out of 8 samples, with a retention time (approx. 8 min.) shorter than that of either of them. Present results indicate a higher ratio of leu- to met-enkephalin than has been previously reported for hippocampus.

In subsequent experiments, the whole hippocampus was rapidly dissected on a cooled plastic block into representative subregions. There regions were individually assayed by HPLC-RIA for content of enkephalins, with both met- and leu-enkephalin being found in each region studied. The pattern of immunoreactivity in the hippocampus may be influenced by the 8 min. HPLC peak, which clearly shares immunological characteristics with both met- and leu-enkephalin. The identity of this substance is not known, but it does not appear to be a direct metabolic product of either met- or leu-enkephalin.

26.15 DISTRIBUTION OF TYROSINE HYDROXYLASE (TH)-, LEU^5-ENKEPHALIN (ENK)- AND SUBSTANCE P (SP)-LIKE IMMUNOREACTIVITY IN THE SEPTAL AREA OF THE RAT. C.M. Gall, Dept. of Anatomy, Univ. of Ca., Irvine, Ca. and R.Y. Moore, Dept. of Neurology, S.U.N.Y., Stony Brook, N.Y.

The peroxidase antiperoxidase immunocytochemical technique was used to localize tyrosine hydroxylase-, enkephalin-, and substance P-like immunoreactivity in the septum of adult Sprague Dawley rats.

The pattern of TH-like immunoreactivity within the septum corresponds with previous descriptions of the distribution of catacholaminergic processes obtained with the glyoxylic acid fluorescent method. Thus, TH immunoreactive axonal groups were observed in the central lateral septal nucleus, at the extreme lateral edge of the caudal lateral septal nucleus and at the perimeter of the septofimbrial nucleus. In the former two areas, the varicose TH-positive axons form terminal 'baskets' encapsulating somata and proximal dendrites.

ENK-like immunoreactivity was seen within both perikarya and axons of well delineated fields of the lateral septum. ENK-positive somata were most prevalent in the central lateral septal nucleus (coextensive with the TH axonal field) although a few labeled perikarya were seen in the septofimbrial nucleus and just ventral to the corpus callosum. Lateral to this cell field a dense plexus of varicose ENK immunoreactive axons can be seen to form distinct pericellular baskets around lateral septal neurons.

No SP-positive perikarya were seen. SP-positive axons were seen in all regions of the septum outside the medial septal nucleus/diagonal band complex with marked variation in density. Most notably, only occasional SP fibers were seen in the zone occupied by the principal ENK-positive axonal plexus. A relatively high density of SP-positive axons was found just dorsolateral and ventrolateral to this clear zone. In these more heavily innervated areas the varicose SP axons form pericellular and, more frequently, dense peridendritic terminal arrays.

Although there is irregular overlap between SP and TH axonal fields, it was consistently observed that 1) the pericellular arborizations generated by SP and ENK axons occupy adjacent, non-overlapping fields; 2) TH fibers do not enter the zones of high ENK fiber density; 3) regions containing ENK-positive neurons are innervated by TH positive pericellular baskets; and 4) all immunoreactive axonal types observed in the extreme lateral septum form pericellular and peridendritic terminal arrays. These observations demonstrate a lamination of axonal systems in the lateral septum, an area which lacks well defined cytoarchitectonic subdivisions.

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26.16 HISTOCHEMICAL LOCALIZATION OF IMMUNOREACTIVE DYNORPHIN IN THE TOAD BRAIN AND PITUITARY, R. I. Cone. Addiction Research Foundation, Palo Alto, CA 94304.

A biologically-active opioid, immunoreactive with antiserum raised against dynorphin-(1-13), was isolated from the brain of the toad, Bufo marinus.[1] This opioid probably has considerable sequence homology with porcine dynorphin and resembles dynorphin in having very high concentrations in the neurointermediate lobe of the pituitary and low sensitivity to antagonism by naloxone in the guinea pig ileum preparation. It is clearly different from porcine dynorphin in that it has a different retention time on HPLC and a somewhat lower opioid activity relative to its immunoreactivity.

This report concerns the localization of immunoreactive dynorphin staining in slices of toad brain and pituitary using a double antibody immunofluorescence method.[2] Fibers stained with an antiserum raised against a C-terminal fragment of dynorphin[3] were observed in the pars ventralis of the tuber cinereum, the median eminence, medial and lateral forebrain bundles, and along the ventral floor of the hypothalamus in the dorsal chiasmatic region. In sagittal sections, immunostained fibers appeared to extend into the zona externa of the median eminence. In the pituitary, intense staining was observed in the pars nervosa and no staining in the pars distalis. Immunostained cell bodies were observed in the pars ventralis of the tuber cinereum in animals which had been given colchicine (50µg) 48 hr prior to killing. Staining of fibers and cell bodies and staining in the pars nervosa could be prevented by coincubation of the antiserum with dynorphin (20µM) but not with leucine enkephalin (20µM). Additional results were obtained using an antiserum raised against dynorphin.[3] However, staining with this antiserum was not as consistent perhaps because some populations of porcine dynorphin antibodies do not recognize dynorphin in the toad.

Additional studies are in progress to establish further the location and specificity of immunostaining.

1. Cone, R.I. & A. Goldstein, PNAS, 79, May, 1982.
2. Weber, E. et. al., PNAS, 79, May, 1982.
3. Antisera were a generous gift of Dr. Eckard Weber.

26.17 DISTRIBUTION OF ENKEPHALIN-LIKE IMMUNOREACTIVITY IN THE RAT SPINAL CORD: AN IMMUNOHISTOCHEMICAL STUDY. A.S. Moskowitz, S.M. Breedlove, A.P. Arnold & J.C. Liebeskind. Psychology Dept. and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Previous work (Elde et al, Neurosci., 1: 349 - 351, 1976; Simantov et al, Proc. Natl. Acad. Sci. U.S.A., 74: 2167 - 2171, 1977; Watson et al, Life Sci., 21: 733 - 738, 1977) has shown that moderate to dense enkephalin-like immunoreactivity (ELI) is present in laminae I, II, V, VII and X of the rat spinal cord, with lower levels of ELI found in the other laminae. In the present study we investigated the light microscopic distribution of leu-enkephalin-like immunoreactivity (LELI) at cervical, thoracic, lumbar and sacral levels of the rat spinal cord.

Adult male and female Sprague Dawley rats were used. Cryostat sections were cut at 10 μm and processed using the indirect immunohistofluoresence method. Adjacent sections were incubated with preabsorbed primary antiserum in order to ensure staining specificity or were stained with thionin in order to determine anatomical levels.

At all spinal cord levels moderate to high numbers of LELI containing fibers and terminals were seen in laminae I, II and V, whereas low to moderate numbers were noted in laminae VII, VIII and IX. At all levels moderate numbers of LELI containing fibers were found in the dorsolateral funiculus.

Rostrocaudal differences were noted in the density of LELI containing fibers and terminals in lamina X. At the thoracic level a few fibers and terminals were noted dorsal and lateral to the central canal. At the lumbar level many diffusely distributed fibers and terminals were seen dorsal and lateral to the canal, with low to moderate levels of fibers and terminals ventrally. At the sacral level a dense concentration of fibers and terminals was found dorsomedial to the canal, with low to moderate levels of fibers and terminals dorsolaterally, laterally and ventrally.

At the L_5 and L_6 levels moderate numbers of LELI containing fibers and terminals were seen in the sexually dimorphic nucleus of the bulbocavernosus (SNB). The SNB is much larger in adult male than in female rats and consists of motorneurons innervating striated muscles involved in male copulatory behavior (Breedlove & Arnold, Sci., 210: 564 - 566, 1980). No differences in the number or intensity of LELI containing fibers and terminals were seen in males and females.

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26.18 REGULATION OF ENDOGENOUS OPIOID PEPTIDE CONTENT IN CULTURED NEURAL CELL LINES. K. M. Braas, S. R. Childers and D. C. U'Prichard. Northwestern Univ. Med. Sch., Chicago, IL 60611, Univ. Florida Coll. of Med. 32610.

Recent reports have demonstrated that numerous neural cell lines contain endogenous opioid peptides. The neuroblastoma x glioma hybrid cell NG108-15 and the neuroblastoma cell N1E-115 have been shown to contain enkephalins (Glaser et al, Eur. J. Pharm. 65:319, 1980; Gilbert, et al, J. Biol. Chem. 257:1274, 1982). We have shown staining of select NG108-15 cells using anti-Met^5-enkephalin (ME) and anti-Leu^5-enkephalin (LE) which is correlated to the state of differentiation induced by dibutyryl cAMP (dBcAMP) treatment. We now describe enkephalin staining in several neural cell lines which possess opiate receptors. The cell lines NG108-15, N1E-115, NCB-20, N4TG-1, N18TG-2 and C6BU-1 were cultured as previously described (Braas et al, J. Histochem. Cytochem. in press, 1982), along with human skin fibroblasts and BHK cells. Two days after plating, some cultures were treated for 5 or 7 days with 1mM dBcAMP. NG108-15 cells grown in serum-supplemented or defined medium were also treated with 1mM 8-bromo-cAMP, 10μM PGE_1 plus 100μM IBMX, or 2mM sodium butyrate (NaB). Further treatment of some cultures with arabinosylcytosine (ARA-C) killed proliferating cells and pure cultures of differentiated cells were obtained. The cells were fixed, processed, and immunocytochemically stained for LM and EM as previously described using anti-ME or anti-LE with the peroxidase anti-peroxidase complex technique. Intense enkephalin-like immunoreactivity was localized throughout the cytoplasm and short processes of select untreated NG108-15, N1E-115, NCB-20 and skin fibroblasts, while C6BU-1 and BHK cells showed little to no staining. Induction of differentiation increased the volume fractions of stained cells. At the EM level, stain was localized to the dense core vesicles found in the cytoplasm and processes of select cells. HPLC analysis and radioreceptor assay demonstrated the presence of 12.6 and 15.1 fmole/10^6 cells of ME and LE in untreated NG108-15 cells grown in serum supplemented medium which approximated the content in cells grown in defined medium. This rose to 30.8 and 33.0 fmole/10^6 cells following dBcAMP treatment. Treatment with ARA-C increased the ME content to 81.0 fmole/10^6 cells, while LE content remained unchanged. Apparently larger increases of enkephalin content was observed for both ME and LE in defined medium cultures treated with NaB. Untreated N1E-115 cells contained 40.7 and 33.5 fmole/10^6 cells of ME and LE respectively. The biochemical analysis for ME and LE correlated well with the immunocytochemical data indicating that the enkephalin content is related to the state of differentiation. Supported by ACS IN-123 (KMB), USPHS grants NS15595 and MH36183 (DCU) and Research Starter Grant from Pharmaceutical Manufacturers Assoc. Found. (SRC).

27.1 BEHAVIORAL EFFECTS OF INDOLE- AND PIPERAZINE-TYPE SEROTONIN RECEPTOR AGONISTS. I. Lucki and A. Frazer. Depts. of Psychiatry and Pharmacology, University of Pennsylvania School of Medicine, and Veterans Administration Hospital, Philadelphia, PA 19104.

The abilities of various indole- and piperazine-type serotonin receptor agonists to alter locomotor activity and to produce the serotonin syndrome were examined in rats.

Locomotor activity of rats was measured in Plexiglas cages, 44x24x20-cm, that employed two photocell detectors to measure a defined ambulatory response of 18 cm along the longitudinal axis of the cage. Intraperitoneal administration of the piperazine-type serotonin agonist, trifluoromethylphenylpiperazine (TFMPP), produced a dose-related decrease in locomotor activity. Activity was reduced significantly by TFMPP at doses of 2.5 mg/kg and higher. The suppression of locomotor activity could be prevented by pretreatment with a number of serotonin receptor antagonists. The potency order for these antagonists for preventing the effects of TFMPP (5 mg/kg) was (most potent to least potent): metergoline mianserin methysergide = cinanserin. Metergoline produced significant blockade of TFMPP's effects at doses as low as 0.3 mg/kg. In contrast, ketanserin and pipamperone, relatively potent and specific antagonists of the serotonin$_2$ receptor, were not capable of preventing TFMPP's effect on locomotor activity at doses as high as 10 mg/kg.

Intraperitoneal administration of indole-type compounds like 5-methoxy-N,N-dimethyltryptamine (5-MeODMT, 3 mg/kg) and d-lysergic acid diethylamide (LSD, 4 mg/kg) to rats produced the serotonin syndrome, a stereotyped behavior frequently used to examine serotonin receptor activation in vivo using rats (B. Jacobs, Life Sci., 1976, 19, 777-786). Pretreatment with metergoline blocked the serotonin syndrome produced by 5-MeODMT. However, TFMPP was not capable of producing the serotonin syndrome at doses up to 36 mg/kg, when toxic effects appeared in the animals.

These results demonstrate differences in the behaviors produced by indole-type and piperazine-type serotonin receptor agonists. It is possible that an interaction of these agonists with different types of serotonin receptors underlies their different behavioral effects.

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27.2 HORMONAL MANIPULATIONS OF THE "SEROTONIN BEHAVIORAL SYNDROME." C.T. Fischette, A. Biegon and B. McEwen . The Rockefeller University, New York, NY 10021.

Sex differences have been found in the stereotypic tryptophan-induced behavioral syndrome (Biegon, A. et al., Psychopharm., 61: 77, 1979), a behavioral pattern which has been correlated with the serotonin$_2$ (5HT-2) receptor (Peroutka et al., Science 212: 827, 1981). In an effort to further characterize the influence of sex hormones upon the serotonergic system, we have used the "serotonin syndrome" as an endpoint to study the effects of hormonal manipulations upon a neurotransmitter system. Pargyline (50mg/kg, i.p.) was administered 1h before l-tryptophan (50mg/kg, i.p.). Animals were observed at 1 and 2h after tryptophan injection and scored for the presence of stereotyped behaviors indicative of 5HT stimulation (Jacobs et al., Life Sci., 19:777, 1976): i.e., resting tremor, head shakes, forepaw treading, hindlimb abduction, salivation, straub tail and hypersensitivity to touch. Results indicate that: 1) a significantly higher number of females exhibit the response as compared to males; 2) ovariectomy has no effect; 3) castration completely abolishes the sex difference; 4) testicular feminized mutants (tfm/y), having a deficit of androgen receptors, respond similarly to females; 5) the sex difference is observed in 18-day old prepubertal as well as adult animals; and 6) this effect is not strain-specific, since it occurs in Sprague-Dawley, King-Holtzman and Wistar rats. The results suggest that androgens, which exert their effects via androgen receptors, reduce the sensitivity of the rat brain to elevated 5HT concentrations. This effect may be mediated by androgen actions on 5HT-2 receptors, although other interpretations are possible. In this connection, we have found that in gonadodectomized male and female rats the pattern of response for 5HT-1 receptor binding in microdissected nuclei differs when animals are given the same hormonal (estrogenic) stimulus (Fischette, C. et al., Fed. Proc., 41(4): 1268, 1982). While further studies on sex differences in the 5HT-2 system are required, current results are consistent with sex differences observed in the occurrence of affective disorders in humans.

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27.3 ACUTE AND CHRONIC EFFECTS OF A NEW ANTIDEPRESSANT, TRAZODONE, ON AN ANIMAL MODEL OF DEPRESSION. J.N. Hingtgen*, H.C. Hendrie* and M.H. Aprison. Institute of Psychiatric Research and Depts. of Psychiatry and Biochemistry, Indiana University School of Medicine, Indianapolis, IN 46223.

Previous data from our laboratories have indicated that acute pretreatment with clinical doses of the antidepressive drugs, mianserin, amitriptyline, imipramine and iprindole, results in the blockade of D,L-5-hydroxytryptophan (5-HTP; 50 mg/kg) induced depression in rats working on a food-reinforced operant schedule (Nagayama et al. Pharmacol. Biochem. Behav. 15, 125, 1981). To distinguish between presynaptic and postsynaptic events, these drug effects were compared to those of fluoxetine, a known specific uptake blocker of serotonin (5-HT), which potentiated the 5-HTP induced depression, and to methysergide, a postsynaptic blocker of 5-HT, which almost completely abolished the depressive effect of 5-HTP. Using the same 5-HTP animal model of depression, we are currently studying the effects of a new antidepressive drug, trazodone, a triazolopyridine compound. Rats working for milk reinforcement and exhibiting behavioral depression following administration of 5-HTP (50 mg/kg; I.P.) were pretreated (1 hr. before the 5-HTP injection) with 1, 2, or 4 mg/kg trazodone with resulting blockade of 5-HTP induced depression of 35, 62 and 70%, respectively. Chronic administration of 2 mg/kg trazodone/day also resulted in a significant blockade of the 5-HTP effect (75%). The blockade of 5-HTP induced depression following both acute and chronic treatment with trazodone was similar to that found in our studies with mianserin and amitriptyline. Since fluoxetine potentiates the behavioral effect of a low dose of 5-HTP, we tested for presynaptic effects of trazodone by giving a lower dose of 5-HTP. Neither 2 mg/kg or 4 mg/kg trazodone was found to potentiate the shorter period of depression following 25 mg/kg 5-HTP. Thus, as with other antidepressive drugs used with the 5-HTP animal model of depression, these data suggest an important postsynaptic mechanism associated with trazodone which could be implicated in the therapeutic effectiveness of this drug. These data, as well as CNS binding data for trazodone, provide additional support for the new hypersensitive postsynaptic serotonin receptor theory of depression (Aprison et al., In: Neuropharmacology and Behavior, Plenum Press, N.Y., 1978; Aprison and Hingtgen, In: Serotonin, Plenum Press, N.Y., 1981). (Supported in part by Association for Advancement of Mental Health Research and Education, Inc., Indianapolis (JNH;MHA) and Indiana Department of Mental Health Grant (HCH) 178-679-005.)

27.4 DIFFERENTIAL EFFECTS OF TWO SEROTONIN DEPLETORS ON APOMORPHINE-INDUCED BEHAVIOR IN RATS. H. Chow*, C. H. M. Beck. Dep't. of Psychology, Univ. of Alberta, Edmonton, Alberta, T6G 2E1.

Para-chlorophenylalanine (p-CPA) and para-chloroamphetamine (p-CA) are both serotonin depletors, however, they have different mechanisms of action. The major difference is that p-CPA blocks new synthesis of serotonin while p-CA irreversibly destroys serotonergic neurons. A challenge of p-CPA and p-CA treated animals with apomorphine (Apo) was used in this study to reveal the behavioral correlates of these differential biochemical effects. Sixty male Sprague Dawley rats, randomly assigned to 6 groups, received one of the following injections 3 days before testing: saline (2 ml/kg, .9%, i.p.) or p-CPA (400 mg/kg, i.p.) or p-CPA (250 mg/kg, i.p.) or p-CA (10.4 mg/kg, i.p.) or p-CA (6.4 mg/kg, i.p.). Five minutes before testing, these rats received injections of either saline (2 ml/kg, i.p.) or Apo (5 mg/kg, S.c.). The rats' behavior while in a wooden test box (56x66x64 cm) was recorded continuously by a trained observer during 18 two minute sessions interpolated over 78 minutes. The behavior was recorded using a list of 14 mutually exclusive and 2 non-exclusive behavioral categories. In half of the sessions the rat was alone and in the other half it was paired with a naive, non-drugged rat. Drug-induced behaviors were collapsed into three composite categories, namely, hyperactivity (speed of locomotion), stereotypy (time spent in gnaw, nod, rear-gnaw), hyperreactivity (frequency of jumping). The expression of neither hyperactivity nor stereotypy was affected by the presence of the partner in the test box. Addition of p-CA (10.4 mg/kg or 6.4 mg/kg) to Apo resulted in significantly more hyperactivity and hyperreactivity when compared to both saline-Apo and p-CPA (400 mg/kg) - Apo groups. Addition of p-CPA (400 mg/kg) to Apo resulted in significantly more time spent gnawing on the test floor while saline with Apo resulted in significantly more time spent gnawing on the side of the test box while rearing when compared to all other groups. The proportion of time spent in stereotypy was similar for the saline-Apo and p-CPA (400 mg/kg) - Apo groups. However, the nod component contributed most significantly to the stereotypy for the saline-Apo group whereas the gnaw component accounted for most of the stereotypy in the p-CPA (400 mg/kg) - Apo groups. Thus, the addition of p-CPA (400 mg/kg) to Apo seemed to have altered only the specific form of the Apo stereotypy. In conclusion, it was possible to behaviorally differentiate p-CPA and p-CA with the Apo challenge.

27.5 VOLTAMMETRY IN VIVO: PHARMACOLOGICAL STUDIES IN THE FREELY MOVING RAT WITH MODIFIED ELECTRODES. C.D. Andrews, K. C. Veneziano* and P. J. Knott. Dept. Pharmacology, Marshall Univ. Sch. Med., Huntington, WV 25701, USA.

Voltammetry in the brain of the freely moving rat enables changes in the release of neurotransmitters and their metabolites to be made over extended periods in conjunction with behavioural measurements. We have developed an automated method for voltammetric recording which offers versatility of control with powerful data storage and analytical capabilities. Timing control and electrode selection is provided by an Apple II+ microcomputer which is interfaced to a DCV-5 voltammetry controller (Bioanalytical Systems Inc.). Using this system remote control of chart recorder, voltammetry controller and connection of appropriate electrodes from multiple implants is achieved by a timing program in BASIC. Chemical modification of graphite-nujol paste electrodes by inclusion of stearic acid (Lane, R.F., Soc. for Neurosci., 1981) improves resolution of the dopamine (DA) peak from ascorbic acid (AA) and dihydroxphenylacetic acid DOPAC) by repulsion of acid groups at the electrode surface. We have used stearate modification of silicone-graphite paste electrodes. In vitro studies suggested that inclusion of silicone oil further improved the DA selectivity.

With electrodes chronically implanted in the striatum or nucleus accumbens, linear sweep voltammetry (20mV/sec) in the behaving rat produced peaks at approximately 140mV, 250mV and occasionally at 415mV. Pharmacological studies suggest that the peak at 140mV reflects DA release. Thus this peak increased with amphetamine (2mg/Kg i.p.) and decreased with γ-butyrolactone (250mg/Kg i.p.), returning to preinjection values after several hours. The monoamineoxidase inhibitor pargyline (75mg/Kg i.p.) caused a gradual but prolonged increase in the peak thought to reflect extraneuronal DA and clearly could not represent either DOPAC or HVA as the decline of these metabolites following monamineoxidase inhibition is well established.

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27.6 MOUSE STRAIN DIFFERENCES IN BEHAVIORAL RESPONSE TO AMPHETAMINE, P. K. Randall* and J. S. Randall*, (SPON: S. S. Erlich) Dept. of Physiology & Biophysics, U. S. C. Sch. of Med., Andrus Gerontology Ctr., Los Angeles, CA 90007.

Neurochemical and neuroanatomical variation between inbred mouse strains are potential model systems for examining brain-behavior relationships. For example, amphetamine- and apomorphine- induced stereotyped behaviors have been used to assess the functional significance of differences in striatal dopaminergic innervation and receptor number between CBA/J and BALB/cJ mice. We have observed that mice, in general, exhibit qualitatively different "stereotyped" behaviors in response to apomorphine and amphetamine, and the strains, in particular, differ qualitatively as well as quantitatively in their responses to each of these drugs. A complete description of the behavioral response to a wide range of amphetamine doses is critical for making quantitative strain comparisons.

CBA/J, BALB/cJ and C57BL6/J mice were observed at intervals of 6 min for 1 hr following IP injections of 2.5, 5.0, 10.0, 20.0 and 40.0 mg/kg d- amphetamine sulfate (n=10/gp). Presence or absence of 28 behavioral categories during each 20 sec observation were recorded. Several behaviors usually contributing to stereotyped behavior rating scales showed striking strain variation.

The dose response curve for locomotor activity and repetitive locomotor behavior suggested an order of sensitivity of BALB/cJ > C57BL6/J > CBA/J. Gnawing and sniffing, however, suggested qualitative strain differences. CBA/J mice increased continuous sniffing as a function of dose through 20 mg/kg. However, neither BALB/cJ nor C57BL6/J mice showed this behavior even at doses as high as 40 mg/kg. Intermittent sniffing decreased with dose in BALB/cJ mice. Gnawing increased with dose in BALB/cJ and C57BL6/J mice but was never observed as a component of the drug response in CBA/J mice. Grooming was only sporadically observed in any strain at 2.5, 5.0, and 10.0 mg/kg. In BALB/cJ mice abortive and self-injurious grooming behavior became the predominant response at 40 mg/kg. This behavior was also observed in CBA/J and C57BL6/J mice at a much lower frequency.

These data are consistent with reports that CBA/J mice are less sensitive to amphetamine than are BALB/cJ mice, but suggest caution in the application of rating scales across these strains.

27.7 ARE BETA 1, BETA 2 OR VASOPRESSIN RECEPTORS INVOLVED INTO DEPRESSION ? B. Delbarre, G. Delbarre, D. Casset-Senon*. Lab. Chir. Exp. Fac. Med., 37032 Tours - France.

Pharmacological and clinical studies suggest that Beta 2 adrenergic stimulant have an antidepressive activity. The mechanism may be explained by release of vasopressin (VP) in blood and C.S.F. (Delbarre et al. Neuroend. lett. 1982 (4) 2). However, a significant decrease in the density of beta adrenergic receptors was observed after antidepressive agents administration (Sulser et al. Biochem. Pharmacol. 1978, 27, 257). In these studies, no distinction has been made between beta 1 and beta 2 receptors. (R.N. Pittman et al. Brain research 1980, 188,357) have shown that beta 1 and beta 2 receptors are differently distributed in C.N.S and appear to be independently regulated. In order to investigate the putative role of these receptors in depression, we have used the antagonism to reserpine induced hypothermia test in mice. Injected in the lateral ventricle (I.C.V.) lysine and arginine VP (0,01 to 0,1 U/mouse), beta 1 selective antagonist agent practolol (20µg/mouse) and intra peritoneal beta 2 stimulant salbuta mol (1 mg/kg) clembuterol (0,5 mg/kg) reverse this hypothermia. But dobutamine (20 µg/mouse) I.C.V, a beta 1 stimulant agent does not reverse hypothermia. As norepinephrine (10 µg/mouse ICV) dobutamine (10 µg/mouse ICV) induces hypothermia. This action is antagonized by DMI (10 mg/kg I.P), practolol (20 µg/mouse ICV) and clembuterol (0,5 mg/Kg IP).

These results suggest that beta 1 and beta 2 receptors independently regulated have also opposite effects on depression and on A.D.H. release.

27.8 MODULATION OF DOPAMINERGIC NIGRO-STRIATAL TRANSMISSION BY PHENCYCLIDINE. H.D. Everist and A. Pert. Biological Psychiatry Branch, NIMH, Bethesda, MD 20205.

There is considerable evidence to suggest that some of the pharmacological effects of phencyclidine (PCP) are mediated through the dopamine system. While PCP has been shown to influence dopaminergic activity, the loci of such actions are unknown. Of interest is the finding that the caudate nucleus as well as the substantia nigra (SN) contain relatively high concentrations of PCP receptors. The purpose of these studies was to ascertain whether the effects of PCP on dopaminergic activity are mediated through either of these structures. In the first series of studies, rats were lesioned unilaterally in the SN with 6-hydroxydopamine. Two to three weeks later the animals were tested in an automated rotometer following i.p. injections of 3, 10 and 20 mg/kg PCP. PCP was found to induce dose-dependent ipsilateral rotational behavior. This effect was attenuated by microinjections of haloperidol (10 µg) into the striatum contralateral to the lesion. In the second series of studies, direct unilateral microinjections of PCP (3, 10 and 25 nmoles) into the SN induced dose-dependent rotational behavior contralateral to the injection. This effect was attenuated by injections of haloperidol into the ipsilateral striatum. Unilateral injections of +PCMP into the SN were five times as effective in eliciting rotational behavior as -PCMP. This corresponds with the efficacy of these two enantiomers in inhibiting PCP binding to brain homogenates. Unilateral injections of PCP (50 nmoles) into the caudate nucleus were without effect on rotational behavior. The findings from these studies suggest that PCP may activate the dopaminergic nigrostriatal pathways through an action in the SN. The precise mechanism of this effect is under study.

27.9 EFFECTS OF CLONIDINE AND NALOXONE ON SELF-STIMULATION RESPONDING BEFORE AND AFTER CHRONIC AMPHETAMINE TREATMENT. Nancy J. Leith, Dept. Pharmacol., Vanderbilt Med. Sch., Nashville, TN 37232

Chronic administration of amphetamine (AMPH) has been shown to produce pharmacodynamic tolerance to the drug's facilitating effects on self-stimulation (SS) responding and a marked post-drug depression of responding reflected in an elevation of the reward threshold (Leith and Barrett, Psychopharmacologia, 46, 1976). The present study was undertaken to provide a first assessment of a possible role for norepinephrine (NE) or opiate systems in the behavioral changes produced by chronic AMPH by examining the effects of clonidine (CLON), an α_2 agonist, and naloxone (NAL), an opiate antagonist, before and after treatment with chronic AMPH.

Animals with electrodes implanted in the MFB were repeatedly tested at 15 current intensities for two successive 15 min sessions. During the first session, baseline responding was assessed. On separate days, subcutaneous injections of AMPH .3 mg/kg, CLON (0.025-.15 mg/kg), NAL (1-4 mg/kg), AMPH + CLON or AMPH + NAL were administered and the second session begin 5 min later. AMPH produced its typical lowering of the reward threshold whereas CLON produced a dose dependent elevation of the threshold with the maximal effect achieved by a dose of 0.1 mg/kg. NAL alone had no effect in the dose range tested. Both CLON and NAL blocked the facilitation produced by AMPH. When acute testing was completed, testing was terminated and increasing doses of AMPH (1-12 mg/kg) were given 3 times daily for 4 days. Two days later the animals were again tested with selected pretreatment doses of the drugs.

The rats developed tolerance to AMPH facilitation of SS responding, which is primarily a reflection of an elevation of the baseline reward threshold. CLON was still able to further elevate the threshold in a dose dependent manner as it had prior to chronic AMPH. Thus, the depressed baseline produced by chronic AMPH is likely unrelated to changes in α_2 receptors.

Testing with NAL, however, did reveal changes after chronic AMPH. Although NAL still had no effect by itself, it no longer blocked AMPH facilitation in chronic AMPH treated rats. When the animals were retested 10 days later and baseline responding had recovered to pretreatment levels, NAL was again able to block the AMPH facilitation. Thus, although AMPH produces a similar relative facilitation of SS responding in acutely and chronically treated rats, the brain mechanisms involved in the two situations must be somewhat different since the facilitation produced acutely is NAL sensitive whereas that obtained in chronically treated animals is not (Supported by MH29217).

27.10 BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF PHENCYCLIDINE AND ITS METABOLITES IN THE CAT. S. Howard-Butcher, M.S. Levine, Dept. of Pharmacology, Mental Retardation Research Center, Brain Research Institute, UCLA, Los Angeles,CA 90024.

We have previously shown that chronic treatment with low doses (1-2 mg/kg) of phencyclidine (PCP) produced marked behavioral effects in the developing kitten (Levine et al., Neuropharmacol. 20: 743, 1981). These effects are age-dependent. Under 21 days the major response consisted of rostrocaudal forelimb movements. After this age the major responses were ataxic locomotion and waxy rigidity. In the present experiment we assessed the behavioral effect of subcutaneous injections of higher doses of PCP, two of its metabolites, phenylcyclohexylamine (PCA) and an alcohol (N-(5-hydroxypentyl)-1-phenylcyclohexylamine) (AL) (Cho et al., Life Sci. 28: 1075, 1981) and one PCP analog (N-N diethylcyclohexylamine (NND)) in kittens between 35-50 days of age. To date we have tested the effects of these substances on 14 kittens from 4 litters. The behavior of the kittens was assessed from 10-15 min pre-injection to up to 7-10 hrs postinjection and rated on a 5 point scale. The most intense response was produced by PCP at both doses and consisted of waxy rigidity (limbs in abnormal postures for extended time periods) with the animal completely immobile. All animals tested displayed waxy rigidity and immobility from 1-2 hrs at 5 mg/kg and for 4-5 hrs at 10 mg/kg. Less intense responses were produced by NND and consisted of locomotor activity with the animal flat on its stomach. PCA was less effective, producing only tremor and staggering followed by loss of hindlimb support. AL was least effective, producing almost no behavioral effect. For all compounds higher doses produced similar behavioral actions as the lower doses but the duration of action was longer.

We have also assessed the capabilities of PCP to release dopamine (DA) in the caudate of 3 adult cats using cyclic voltammetry. Low doses of PCP (1 mg/kg subcutaneous) consistently produced an increase in release of caudate DA (3-5 times control values). The increase in release occurred within 5 min postinjection and continued for at least 3 hrs. Subsequent injections 1 and 2 weeks later did not produce consistent increases in caudate dopamine release suggesting that a rapid tolerance may have developed.

These results indicate that some of the metabolites of PCP, while active, are not as potent as PCP itself. Additionally, our results provide support for a role of caudate DA in the mechanism of action of PCP.

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27.11 AMANTADINE BLOCKS THE EFFECTS OF SEVERAL CENTRAL STIMULANTS, BUT NOT THOSE OF ALCOHOL AND PENTOBARBITONE, IN MICE. M.K. Menon and V.G. Haddox*. Psychopharmacol. Res. Lab. and Alcohol Treatment Unit, V.A. Med. Ctr, Sepulveda, CA 91343 and Dept. Psychiat., UCLA School of Medicine, Los Angeles, CA 90024.

We reported earlier that amantadine blocks the central stimulant effects of d-amphetamine in mice (Menon et al., Eur. J. Pharmacol. 21:311, 1973). This paradoxical effect was confirmed in rats by others (Clark et al., Proc. Soc. Exp. Biol. Med. 151: 434, 1976). Amantadine also reduced the antiparkinsonism effect of l-dopa in humans, but only when given before the latter (Cox et al., J. Neurol. Neurosurg. Psychiat. 36:354, 1973). Experiments were performed to know whether amantadine is able to block the locomotor stimulant effects of drugs other than d-amphetamine.

Male Swiss mice were used and all the drug treatments were made intraperitoneally. The test groups received amantadine HCl (150 mg/kg) 120 min before the stimulant drug. Control mice were treated with distilled water in place of amantadine. One hr after either amantadine or water injection, the mice were placed in individual cages for familiarization and the stimulants were administered after a further 1 hr. A Varimex activity meter was used and the activities were recorded on a printing counter. Differences in the 60 min activity readings between the test and control groups were used for calculating the percent change in their locomotor effects.

Amantadine pretreatment caused a marked reduction (71.5% blockade) in the response to d-amphetamine. This confirmed our earlier findings. Amantadine treatment also was very effective in blocking the responses of mice to other stimulants such as methylphenidate (20 mg/kg, 70.2%), amfonelic acid (1 mg/kg, 91.9%), caffeine (20 mg/kg, 42.9%), 1,3-dimethyl-5-aminoadamantane (D-145, 20 mg/kg, 71.4%), phencyclidine (10 mg/kg, 58.7%), cocaine (50 mg/kg, 69.6%) and d-deprenyl (20 mg/kg, 77.8%). The levorphanol-induced stimulation was not modified by amantadine. Amantadine also did not reduce the duration of the "sleeping-time" caused either by alcohol (5 g/kg) or pentobarbitone (60 mg/kg). The value of amantadine in the management of stimulant abuse needs to be explored. (Supported by the Veterans Administration).

27.12 TL-99 AND 3-PPP, DIFFERENTIAL ACTION IN PRODUCING CONTRAVERSIVE TURNING IN THE 6-OHDA-LESIONED RAT. Gregory E. Martin and R. J. Bendesky*. Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

TL-99 (Goodale et al., 1980, Science, 210:1141) and 3-PPP (Hjorth et al., 1980, Psychopharm. Bull. 16:85) are two recently reported agents said to be selective for the dopamine autoreceptor. Although TL-99 was thought to qualify as a selective DA autoreceptor agonist due to the fact that it did not produce contraversive turning in the 6-OHDA-lesioned rat (Goodale et al., 1980), a recent report demonstrated TL-99 did produce contraversive turning but the dose-response curve had an inverted U shape (Martin et al., 1981, EJP, 76:15-23). The purpose of the present study was to extend our previous work by quantifying the number of turns produced by each autoreceptor agonist given over a wide dose range. Since TL-99 also possesses potent α_2 activity (Hicks and Cannon, 1980, J.P.P. 32:786), the effect of pretreatment with yohimbine on the TL-99- or 3-PPP-induced turning was also examined. A unilateral 6-OHDA lesion was created in the substantia nigra of 120 female Sprague-Dawley rats using standard stereotaxic procedures. Yohimbine was administered s.c.; all other drugs were given i.p. Turns were recorded in automated rotometers for 1-2 hr following drug administration. TL-99 was given in doses of 1, 1.5, 2, 3, 4 and 6 mg/kg, 3-PPP was given in doses of 1, 3 and 9 mg/kg and apomorphine in doses of 0.1, 0.5 and 1.0 mg/kg. Each dose of each drug was given to 8 rats. Yohimbine (0.5 mg/kg s.c.) was given 1 hour before TL-99 or 3-PPP using a counterbalanced design in which each animal served as its own control. 3-PPP produced a dose-related turning response ($\bar{x} \pm$ S.E.M. = 172 $\pm$ 95 tph after 1 and 564 $\pm$ 128 tph after 9 mg/kg) as did apomorphine (210 $\pm$ 67 tph after 0.1 and 519 $\pm$ 49 tph after 1.0 mg/kg). The turning produced by TL-99 was dose-related up to 3 mg/kg (39 $\pm$ 9 tph after 1 and 220 tph after 3 mg/kg), but the number of turns declined as the dose level was further increased, viz 195 $\pm$ 35 and 122 $\pm$ 31 after 4 and 6 mg/kg, respectively. Pretreatment with yohimbine produced a significant increase (from 130 $\pm$ 15 to 240 $\pm$ 49 and 95 $\pm$ 11 to 218 $\pm$ 45 tph) in TL-99 (4 and 6 mg/kg respectively)-induced turning. Turning produced by 3-PPP (1.0 or 3.0 mg/kg) was not significantly altered by yohimbine pretreatment. Both 3-PPP and TL-99 produce a vigorous contraversive turning response in 6-OHDA-lesioned rats but the sedation produced by the α_2 properties of TL-99 produces a fall in potency of TL-99 at higher dose levels. Hence, 3-PPP appears to be the more selective dopamine agonist. The 6-OHDA-lesioned rat model does not distinguish between post synaptic and DA autoreceptor agonists.

27.13 PHENYLETHYLAMINE DISRUPTS LATENT INHIBITION IN THE GERBIL, Charles J. Hannan, Jr., Robert Patterson*. Clinical Investigation Dept., Eisenhower Army Medical Center, Ft Gordon GA. 30905

The gerbil is used to study attention employing the latent inhibition paradigm. Briefly, the latent inhibition paradigm involves exposing one of two groups of animals to an innocuous stimulus, we used a tone,and then testing both groups in a conditioned avoidance response (CAR) situation using for the conditioned stimulus (CS) the tone previously used, paired with the unconditioned stimulus (US), electric shock. Animals which were exposed to the unpaired CS do not perform as well in the CAR as animals that had never experienced the CS. The explanation for this could be that in the CAR situation the previously meaningless CS had to be unlearned before the meaningfulness of the CS-US relationship could be learned. An animal not exposed or not attending to the CS would only have to learn the CAR and therefore would learn more quickly than its' familiarized counterpart. It was recently reported that amphetamine disrupts latent inhibition; therefore both familiarized and unfamiliarized animals perform at the same level in the CAR (Solomon et al, Biol Psychiat 16:519-537,1981). The same investigators also demonstrated a protective effect of chlorpromazine on the amphetamine disrupted latent inhibition. The authors propose latent inhibition disruption by amphetamine as a model for the attentional defect associated with certain schizophrenias. Phenylethylamine (PEA) has been called an endogenous amphetamine and elevated concentrations have been found in some schizophrenics, so evaluation of its' effects in the latent inhibition model seemed appropirate. The methodology used was similar to that reported in the above reference. PEA was administered for at least 10 days at 50 mg/kg ip each day. Animals were then tested in a BRS/LVE shuttle box.

LATENT INHIBITION MEASURES (MEAN ± SE)

GROUPS (n)	AVOIDS CONTROL/PEA	TRIALS CONTROL/PEA
FAMILIARIZED (5)	30±4 30±12	60±3 11±2
UNFAMILIRIZED (5)	47±2 30±8	27±5 20±10

Saline treated controls exhibit the expected effects of familiarization with the CS: fewer avoids and a greater number of trials to criterian (4 avoids out of 5 trials), relative to the unfamiliarized group. PEA disrupted the latent inhibition effect, as amphetamine was reported to do and may produce a syndrome more consistent with human schizophrenia. Further studies are underway to evaluate the effect of antipsychotic agents in this model.

27.14 POTENTIATION OF THE BEHAVIOURAL EFFECTS OF THE ANTIDEPRESSANT, PHENELZINE, BY DEUTERIUM SUBSTITUTION. K.M. Dewar*, L.E. Dyck*, A.A. Boulton and C.T. Dourish* (Sponsor: J.D. McQueen). Psychiatric Res. Div., University Hospital, Saskatoon, Sask., S7N OXO, Canada.

Recent studies in this laboratory have demonstrated that deuteration of β-phenylethylhydrazine (phenelzine, PLZ) in the α,α,β,β-positions of its side-chain enhances its ability to inhibit MAO after systemic administration. To determine whether the presence of deuterium in these positions affects the behavioural responses to PLZ, we have compared the effects of intraperitoneal PLZ to those of deuterated PLZ (d_4 PLZ).

Male Wistar rats were individually tested in plexiglass cages positioned in an automatic activity recording device which recorded horizontal and vertical movements via a microprocessor and microcomputer system. In addition, the behaviour of each animal was observed and rated during 15 min of each hour for a 12-hour period by a trained observer. The behaviour of rats given 12.5 or 25 mg/kg of PLZ was similar to that of controls. In contrast, these doses of d_4 PLZ elicited wet dog shakes (WDS), forepaw padding, sniffing, repetitive grooming and hyperactivity. A maximum of 10 and 25 WDS (per 15 min) respectively, 6-7 hours post-injection was observed. Maximal stimulation of horizontal activity was observed after 25 mg/kg. PLZ at a dose of 50 mg/kg produced WDS, splayed hindlimbs, sniffing, repetitive grooming and increased horizontal activity. The latency of onset of behavioural stimulation was 2 hours post-injection and peak effects were observed 6-7 hours after drug administration. A maximum of 12 WDS was observed 6-7 h post-injection. After an equivalent dose of d_4 PLZ, however, a maximum of 26 WDS was observed. The intensity of repetitive grooming, horizontal activity, splayed hindlimbs and sniffing was also enhanced. In contrast to PLZ, d_4 PLZ produced forepaw padding.

Behavioural stimulation was increased quantitatively by deuteration though there was little or no effect on the time course of drug action. The present results are compatible with the finding that deuterium substitution of PLZ enhances its MAO inhibitory properties. Since phenelzine is a clinically effective antidepressant, it appears possible that deuterium substitution may have therapeutic value in the treatment of depression.

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27.15 ELECTROPHYSIOLOGICAL DEMONSTRATION OF BOTH α_2-AGONIST AND ANTAGONIST PROPERTIES OF RX 781094. J.M. Goldstein, L. C. Knobloch* and J.B. Malick. Pharmacology Department, Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, DE 19897.

The effects of RX 781094 (2-[2-(1,4-Benzodioxanyl)]-2-imidazoline), an agent that has been reported to be a potent and selective α_2-adrenoceptor antagonist (Chapleo et al., Brit. J. Pharmacol. 74:842, 1981) on the firing rate of brain norepinephrine neurons in the locus coeruleus (LC) were studied using extracellular single unit recording techniques. Intravenous injection of low doses of RX 781094 ($\leq$0.25mg/kg) produced an unexpected decrease in LC unit activity similar in magnitude to that observed after clonidine (10 µg/kg, iv). This effect was readily reversed with the α_2-antagonist yohimbine (0.5 mg/kg, iv). Low doses of RX 781094 also antagonized the increase in LC unit activity produced by the α_1-antagonist WB4101 (0.1 mg/kg, iv); clonidine also reversed the increase in LC firing produced by WB4101. The ability of RX 781094 to suppress both spontaneous LC unit activity and the enhancement induced by WB4101, and the ability of yohimbine to reverse these effects suggests that at low doses RX 781094 possesses α_2-agonist properties.

When higher doses of RX 781094 were tested ($\geq$0.5 mg/kg, iv), potent α_2-antagonist properties were clearly demonstrated. RX 781094 produced increases in LC unit activity (comparable to yohimbine at 0.5 mg/kg, iv) and markedly elevated the dose of clonidine needed to suppress LC unit activity (from 10 µg/kg, iv to more than 100 µg/kg, iv). In addition, RX 781094 readily reversed the decrease in LC unit activity produced by clonidine. In fact, in the same rat, it was possible to demonstrate both a decrease in LC unit activity (with low doses of RX 781094) as well as an increase in LC unit activity (with higher doses of RX 781094). In comparison, yohimbine, even at doses clearly below those which enhance LC unit activity, did not produce any significant decreases in firing rates.

On the basis of these results, RX 781094, in addition to its known α_2-antagonist properties, also possesses clonidine-like α_2-agonist activity in this model. Thus, RX 781094 may be a partial agonist at α_2-adrenoceptors in the CNS.

27.16 AMPHETAMINE BUT NOT TRH DISRUPTS PERFORMANCE ON THE RADIAL ARM MAZE. Gregory Lucas Belenky, Lysaida Cardenales*, Lydia E. Robles*, David Arday* and John W. Holaday. Dept. Med. Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012.

In preliminary studies amphetamine but not thyrotropin releasing hormone (TRH) disrupted performance on the radial eight arm maze. Two groups of 20 Sprague-Dawley rats each were trained to criterion performance on a radial arm maze. To prevent response chaining, the rats were confined for 5 seconds in the center of the maze after completing each run down an arm. One group of rats was tested with amphetamine, and the other group of rats with TRH. For each drug, three doses were compared against a saline control: 0.5, 1.0, and 2.0 mg/kg amphetamine, and 4.0, 8.0, and 16.0 mg/kg TRH. The rats were tested 15 minutes after injection. Our experiment was conducted over four days using a latin square design such that each animal received all four doses of drug during the course of the experiment. Amphetamine increased running time [$F(3,48)=3.09$, $p=.036$] and decreased number of correct responses [$F(3,48)=2.86$, $p=.046$] in a dose dependent manner. In contrast, these doses of TRH had no effect upon either running time or upon number of correct responses. The total number of responses was unaffected by either drug. Work by others indicates that both amphetamine and TRH stimulate locomotor activity, although at the doses which we used amphetamine resulted in greater analepsis than TRH (Vogel, R.A et al, J. Pharmacol. Exp. Ther. 208(1979)161-168). Further experiments with both drugs are being pursued at higher doses and at longer post-injection delays. Our preliminary results suggest that TRH may differ qualitatively from amphetamine in its stimulatory effects.

27.17 CLONIDINE POTENTIATES DRUG INDUCED SELF-INJURIOUS BEHAVIOR IN RATS. K. Mueller. Department of Pediatrics, Univ. Calif. - San Diego, La Jolla, CA, 92093

Self-injurious behavior is a disturbing component of several syndromes and often occurs in the general retarded population. Self-biting (SB) in rats is an unusual behavioral effect of certain central drugs and may provide clues to the proximal mechanism of self-injurious behavior in humans.

Daily administration of caffeine and implantation of continuous release amphetamine pellets produce reliable SB in rats. Although caffeine and amphetamine are structurally dissimilar and produce dissimilar patterns of behavioral changes, the SB produced by both drugs is similar in several respects: latencies to SB are similar, targets of SB are similar, SB occurs in absence of stereotypy, and incidence of SB is potentiated by clonidine, an α noradrenergic agent.

Long Evans hooded rats (N = 14 for each group) were given daily subcutaneous injections of caffeine 30 min after pretreatment with various doses of clonidine, or were implanted subcutaneously with silicone pellets containing 47 mg amphetamine base. Pellet rats were injected with various doses of clonidine twice daily.

As little as 0.025 mg/kg clonidine produced SB when combined with a subthreshold dose of caffeine (for producing SB in these rats); 0.5 mg/kg clonidine increased the incidence of SB to over 50%. Amphetamine pellet rats were less sensitive to this effect of clonidine; only the highest dose (0.5 mg/kg) potentiated SB. The high dose of clonidine (0.5 mg/kg) reduced the general activity of both caffeine and amphetamine pellet rats; that is, clonidine reduced the frequency of such behaviors as locomotions (caffeine and amphetamine), wet dog shakes, and digging/burrowing (caffeine). However, activity generally remained higher than that of undrugged animals.

There was little evidence of opposing behavioral effects of clonidine at doses selected to produce either predominantly presynaptic (0.01 to 0.05 mg/kg) or postsynaptic (0.5 mg/kg) effects. Clonidine is known to reduce caffeine induced behaviors referred to as the quasi-morphine withdrawal syndrome; its concurrent ability to potentiate SB raises questions both about the central effects of clonidine and the central mechanisms of self-injurious behavior.

27.18 ACUTE LOW-LEVEL MICROWAVE IRRADIATION AFFECTS THE ACTIONS OF PSYCHOACTIVE DRUGS. H.Lai*, A.Horita*, C.K. Chou*and A.W.Guy* (SPON: K. Chan). Dept. Pharmacology,Psychiatry & Behavioral Sciences and Rehabilitation Medicine, University of Washington School of Medicine, Seattle, WA 98195.

Male Sprague-Dawley Rats were exposed to circularly polarized 2450 MHz microwave(2 μs, 500 pps) with an averaged power density of 1 mW/cm^2 (SAR = 0.6 W/kg) for 45 minutes. They were then immediately injected with either apomorphine, d-amphetamine, ethanol or morphine, and physiological and behavioral responses were studied. Apomorphine-elicited stereotypic behavior and hypothermia were significantly enhanced by the microwave irradiation when compared with the responses of sham-irradiated control animals. Amphetamine-elicited hyperthermia and ethanol-elicited hypothermia were significantly attenuated by the irradiation. In the case of ethanol, only the initial fall in body temperature was affected. Both morphine-elicited catalepsy and lethality were significantly enhanced by the acute low-level microwave irradiation. A higher percentage of the irradiated animals showed cataleptic response and died after injection of lower dose of morphine. The temperature response curve to morphine was also altered by microwave irradiation.

27.19 SITE SPECIFIC DIFFERENTIATION OF IDENTICAL BEHAVIORS INDUCED BY d-AMPHETAMINE (Amph) AND PHENYLETHYLAMINE (PEA). B.I. Diamond, A. Hitri*, K.S. Rajan and R.L. Borison. Psychiatry Department, Medical College of Georgia and Downtown V.A., Augusta, GA, 30912 and Illinois Institute of Technology Research Institute, Chicago, IL, 60607.

Previously we have shown that Amph and PEA induced behavioral stereotypy, although phenomenologically similar, can be differentiated by dopamine (DA) blockers that affect predominantly limbic areas, from those that affect striatal areas. Moreover, the fact that PEA is an endogenous amine highly concentrated in limbic areas of the brain, supports its role as a mediator in this behavior which serves as an animal paradigm for schizophrenia. Further evidence is presented in the present studies to support the interaction and differentiation of Amph and PEA stereotypy with DA systems. Male Sprague-Dawley rats (200-250 g) were treated daily with either PEA (50 mg/kg) or Amph (3.75 mg/kg) intraperitoneally for three weeks. Stereotyped behavior was quantitated on a weekly basis by using a linearly increasing rating scale to reflect weekly movement changes. Animals were sacrificed at three weeks, brains removed, and the caudate nucleus and nucleus accumbens dissected. Metal levels for Cu, Zn and Mn were measured by atomic absorbtion spectrometry after digestion of tissues with nitric acid. Levels of DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine (NE) and 5-hydroxyindoleacetic acid (5HIAA) were also measured by HPLC using an electrochemical detector. Receptor binding studies using ^{3}H-spiroperidol as the ligand for DA receptors was performed according to the modified method of Fields et al. (1977). Three week treatment with PEA or Amph did not affect striatal Zn levels, whereas striatal Mn levels were elevated by PEA and Cu levels decreased by Amph. Moreover, chronic Amph elevated striatal DA, DOPAC and HVA levels, without affecting their levels in accumbens tissue. Chronic PEA also increased striatal levels of DA, DOPAC and HVA, which in contrast to Amph, also occured in accumbens tissue. Control animals displayed two striatal binding sites, which were differentially affected by Amph. Treatment with Amph decreased the affinity of the high affinity site, while increasing the numbers of low affinity sites, whereas PEA affected only the low affinity site, decreasing the receptor density. These results support the contention that although PEA and Amph produce nearly identical behavior in animals, their underlying mechanisms of action on different dopaminergic systems of the brain can be separated.

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27.20 CORTICOSTERONE BINDING IN THE CAUDATE-PUTAMEN: MODULATION OF AMPHETAMINE-INDUCED STEROTYPIC BEHAVIORS BY CORTICOSTERONE INTERVENTION. C. H. DeFiore and B. B. Turner. Depts. of Psych. and Biology, Virginia Tech, Blacksburg, VA 24061.

Although corticosterone (CORT) is selectively bound by discrete areas of the rodent brain, particularly the hippocampus (HC), autoradiographic data indicate no binding of CORT in the caudate-putamen (C-P). However, cats pretreated with glucocorticoids have been reported to show a selective increase in high affinity choline accumulation in the C-P. We wished to evaluate the possibility that this observed increase in choline uptake is mediated by glucocorticoid receptors within the C-P.

We asked whether glucocorticoid receptors in the C-P of adrenalectomized (ADX) rats can be detected by biochemical assay. We determined the amount of specific 3H-CORT binding in C-P cytosols and the ability of C-P nuclei to retain 3H-CORT in vivo. Results indicated that the C-P has 56% of the binding capacity of the HC (205 $\pm$ 11 vs 368 $\pm$ 22 fm/mg protein). The apparent K_d of CORT in the two tissues was similar. No difference in the affinity of any of 8 steroids tested relative to CORT was found between C-P and HC. Nuclei purified from the C-P demonstrated about 40% of the radioactivity present in HC nuclei (1,158 $\pm$ 24 vs 2,988 $\pm$ 320 fm/mg DNA).

We then asked whether the increase in striatal choline uptake induced by glucocorticoids has behavioral significance. Stereotypic behaviors are modulated by the action of amphetamine on striatal dopamine neurons and increased cholinergic function within the striatum antagonizes the stimulation of these behaviors by amphetamine. To test the hypothesis that CORT can modulate striatal acetylcholine function, a 3 x 4 factorial design was used: 4 levels of steroid condition (ADX, intact, ADX + 5 mg/kg and 12.5 mg/kg CORT) and 3 levels of amphetamine condition (0, 1.25, and 2.5 mg/kg). Ambulation, rearing and stereotypy were scored over a 90 min period for each rat (n=8). ANOVA indicated significant CORT X amphetamine X time interaction for all behaviors ($p<.001$). Analysis of group differences showed that, although CORT condition was without effect on the saline group, CORT did affect ambulation, rearing and stereotypy in both amphetamine groups. The effect of amphetamine on stereotypy was facilitated by ADX ($p<.01$, 1.25 mg/kg; $p<.05$, 2.5 mg/kg), and antagonized by high CORT replacement ($p<.01$, 1.25 mg/kg; $p<.01$, 2.5 mg/kg). The stereotypy ratings of the group receiving the lower CORT replacement dose resembled the intact group.

These data provide evidence that 1) functional glucocorticoid receptors exist in the rat C-P and 2) that glucocorticoids can antagonize behaviors mediated by amphetamine, possibly by enhancing cholinergic function within the striatum. (Supported by NIH grant 2-S07-RR-07095-13).

28.1

WITHDRAWN

28.2 ACUTE EXPOSURE TO 2.70 GHz PULSED MICROWAVE RADIATION AFFECTS NEITHER PENTYLENETETRAZOL SEIZURES NOR CHLORDIAZEPOXIDE PROTECTION AGAINST SUCH SEIZURES. B. A. Pappas, H. Anisman and R. Ings*. Dept. of Psychology, Carleton University, Ottawa, Ontario K1S 5B6.

One reported psychopharmacological effect of pulsed microwave radiation (PMR) is modulation of behavioral effects of benzodiazepines. These and other findings indicating that PMR affects seizure susceptibility in audiogenic seizure prone rats are consistent with an effect of PMR on brain inhibitory, possibly GABA ergic neurotransmission. We assessed this possibility by examining the effect of brief exposure to PMR upon pentylenetetrazol (PTZ) induced seizures and upon the efficacy of chlordiazepoxide (CDZ) for attenuating such seizures.

In experiment one, relatively unrestrained rats were exposed for 30 min to PMR at power density levels of 0, 5, 10 or 15 mW/cm^2. After exposure they were injected with 0, 20, 40, 60, 70 or 80 mg/kg of PTZ. All three radiation power densities significantly elevated core temperature. PMR did not, however, alter the dose response curve relating seizure parameters and PTZ dose. Exposure to the highest radiation density (i.e. 15 mW/cm^2) very slightly but significantly shortened the latency to seizure onset and also very slightly but significantly increased seizure intensity. Since core temperature elevations were greatest for this PMR exposure group, this effect of PMR on seizures may have been secondary to temperature elevation.

Experiment two assessed the effects of 30 min exposure to these same microwave power densities upon the efficacy of 0, 2.0, 7.5 and 15.0 mg/kg of CDZ for attenuating seizures elicited by 70 mg/kg PTZ. PMR counteracted the hypothermic effects of CDZ in a dose related manner but did not consistently affect the anti-seizure efficacy across all doses of CDZ. There was, however, a suggested enhancement of the anti-seizure effect of only 7.5 mg/kg CDZ by the highest (15 mW/cm^2) radiation power density.

The third experiment aimed to replicate these suggested effects of this (15 mW/cm^2) and higher (20 mW/cm^2), hyperthermia-inducing levels of PMR upon susceptibility to PTZ seizures and the anti-seizure efficacy of CDZ. A more rigorous protocol was employed where the experimenter rating seizures was blind to radiation power density. We failed to repeat the effect of PMR upon either of these phenomena. We conclude that the two isolated, significant effects observed in the first two experiments represented Type II errors (i.e. incorrect rejections of the null hypothesis) which can be expected to occur in large scale experiments. We also concluded from these experiments in which PMR consistently affected neither PTZ nor CDZ action, that a single brief exposure to such radiation has no significant pharmacological effect upon GABA neurotransmission. Supported by Department of National Defence (Canada) contract (B.A.P. and H.A.)

28.3 FURTHER EVIDENCE THAT GABA RECEPTORS MEDIATE THE HYPERKINETIC EFFECT OF INTRARAPHE MUSCIMOL. S. M. Sainati, H. K. Kulmala, and S. A. Lorens. Inst. of Neuropharmacology, Loyola Univ. School of Med., Maywood, IL 60153.

We previously reported (Neurosci. Abstr. 7:925, 1981) that intraraphe microinjections of muscimol (0.44-1.75 nmole in 0.5 µl saline) produced dose-dependent increases in locomotor activity. Intraperitoneal injections of chlordiazepoxide (CDP, 3.8 mg/kg) potentiated, whereas bicuculline (1.1 mg/kg) blocked the muscimol-induced hyperactivity. In order to determine whether these effects of CDP and bicuculline were due to their binding to the same neuronal membranes as muscimol, the following experiments were performed. Cannulae were implanted in the median raphe nucleus (MR) of male albino rats (300-500 g) and the animals adapted to photocell activity chambers. Intra-MR dose-response curves for the water-soluble benzodiazepines, CDP, flurazepam, and midazolam were performed. Drugs were administered in doses of 0.0, 0.22, 0.44, 0.88 and 1.75 nmole in 0.5 µl saline. Both midazolam and flurazepam produced hyperactivity which was most prominent within the first 30 min post-injection. Flurazepam, furthermore, proved twice as potent as midazolam. In contrast, CDP was without effect at any of the doses tested. This observation supports the view that CDP is a "prodrug" which must be demethylated or further modified to form an active metabolite.

In a second experiment, animals received either saline or a sub-effective dose (0.22 nmole) of flurazepam or midazolam into the MR 5 min prior to either a sub-effective dose of muscimol (0.22 nmole) or saline. Only the combinations of a benzodiazepine plus muscimol produced hyperactivity. These combinations, moreover, produced effects as robust as those of a 4-fold higher dose of muscimol alone (0.88 nmole).

Finally, animals received either saline or bicuculline methiodide (0.88 nmole). Bicuculline completely blocked the hyperactivity effects of muscimol. Taken together, these data suggest that the hyperkinetic effects of intra-MR muscimol are due to activation of GABA receptors within the midbrain raphe, rather than at distant sites.

This conclusion is supported by autoradiographic evidence which shows that following intra-raphe injection, 3H-muscimol does not spread appreciably from the target site.

28.4 ACUTE EFFECTS OF GAMMA-HYDROXYBUTYRATE ON TONIC IMMOBILITY IN THE RABBIT.† R. Godbout* and R. T. Pivik, Lab.Neurophysiology, School of Psychology and Department of Psychiatry, Faculty of Health Sciences, University of Ottawa, Ottawa, Ontario K1N 5C8, Canada.

Gamma-Hydroxybutyrate(GHB)is a short-chain fatty acid present in the mammalian brain which is thought to be a metabolite of GABA. Administration of low doses of GHB has been reported to induce sleep and to enhance amounts of paradoxical sleep. At doses exceeding hypnotic levels, GHB has been reported to increase motor activation to the point of inducing seizure activity.

The purpose of the present study was to examine the effects of GHB on the duration, ease of induction and electrographic correlates of tonic immobility (TI), a state variously characterized as a condition of hypnotic sedation and as an innate fear reaction. Previous studies have indicated increased TI duration in conjunction with decreased dopaminergic and cholinergic activity. Since GHB is thought to effect a decrease in neurotransmission based on these neurotransmitters, it was postulated that this compound would facilitate TI.

Five adult New Zealand white male rabbits were chronically implanted (EEG, EMG, EOG) for electrographic recordings, and experimental procedures began after one week of post-operative recovery. TI induced by rapid inversion and placement of the rabbit in the V-shaped trough, was examined under the following conditions: GHB injections at doses of 300, 650 and 1000 mg/kg, a saline injection, and a non-injection (baseline) condition. All injections were administered IV. Sessions were distributed according to a Latin square design and were divided into three time blocks (0, 40 and 120 minutes post-injection). At each time block 5 inductions of TI were attempted. Electrographic recordings were performed periodically.

At the 1000 mg/kg dose level, animals did not exhibit normal characteristics of TI, but instead were extremely flaccid with eyes closed. Accordingly, data from this dosage level were not included in data analyses. For all other conditions, rabbits exhibited normal TI characteristics. No statistical differences were found between conditions for any time blocks for either duration of TI or induction failure rate. However, durations were shown to be longer in GHB sessions vs. non-drug sessions ($p<0.1$).

The present results support previous work indicating that reductions in dopaminergic and cholinergic activities facilitate TI duration.

†supported by the Ontario Mental Health Foundation and MRC.

28.5 DIAZEPAM AND AMINO ACID RELEASE. F. Petty[1,2], A.D. Sherman[1]* and J. Mott[1]*. [1]Neurochemical Research Laboratory, Department of Psychiatry, University of Iowa, Iowa City, IA 52242 and [2]Veterans Administration Medical Center, Iowa City, IA 52242.

The release of endogenous amino acids from slices of frontal cortex was investigated in relation to footshock and to previous experience with footshock. Rats were given 40 minutes of pulsed, intermittant footshock on day 1. On day 2, being placed back into an identical shock box was sufficient to elevate glutamate release by over 50%. Shocking these animals while in the shock box elevated glutamate release only by an additional 10% suggesting that psychological factors were involved in the elevated release. In animals with no previous exposure to shock, being placed in the box or being exposed to mild shock had no effect on glutamate release. Forty minutes of pulsed random footshock produced an increase of over 250%, but this effect had dissipated by 24 hours.

After the initial forty minutes of shock, replacement in the box one, two, or four days later still elevated glutamate release significantly, but replacement at eight days failed to produce the effect. The increased glutamate release could be blocked by diazepam, but not by a sedating dose of haloperidol. In addition, restraint stress sufficient to elevate plasma hydrocortisone levels significantly failed to increase glutamate release.

These data suggest that, in the frontal cortex, glutamate release may be an indicator of anxiety or fear. Since this measure was blocked by diazepam but not haloperidol, it may also be involved in the anxiolytic actions of diazepam.

28.6 EFFECT OF MELATONIN AND Ro 15-1788 ON DIAZEPAM DISCRIMINATION. P. B. Silverman. Neurochem-Neuropharm. Research Section. Texas Res. Inst. Mental Sci., Houston, TX 77030.

Adult male Sprague-Dawley rats were food deprived to about 80% of their free-feeding weight. They were then trained to bar-press for food pellets in a two-lever operant box. Fifteen minutes prior to each daily 20 min. session, rats were injected intraperitoneally (i.p.) with 2.0 mg/kg diazepam or with diazepam vehicle. Following diazepam injection, reinforcement was available for operating one of the levers, while vehicle injection was always paired with reinforcement on the other lever. In either training condition, rats had to emit 20 responses on the appropriate lever in order to obtain a food pellet (FR-20 schedule). Once rats reliably discriminated diazepam from vehicle as indicated by their lever choice for the first twenty responses, the ability of compounds to substitute for (generalize with) diazepam or block the diazepam stimulus was tested. The most interesting results were obtained with melatonin and Ro 15-1788. Melatonin by itself showed no similarity to diazepam at up to 32 mg/kg. It did, however, shift the diazepam dose-response curve appreciably to the left. Initial attempts to train rats to discriminate melatonin from its vehicle have thus far been unsuccessful.

Ro 15-1788 effectively blocked diazepam discrimination. This effect was, however, of limited duration at the doses tested, i.e., Ro 15-1788 was more effective when given as a post-treatment than as a pre-treatment. When tested with pentobarbital, rats responded primarily on the lever paired with diazepam in training. This generalization of pentobarbital with diazepam was not blocked by Ro 15-1788 treatment. Selective antagonists of benzodiazepines such as Ro 15-1788 may prove useful in distinguishing benzodiazepine from non-specific sedative effects of various compounds.

28.7 MECHANISMS OF THE INITIAL TREATMENT PHENOMENON TO DIAZEPAM IN A CONDITIONED SUPPRESSION PARADIGM. D.J. Mokler* and R.H. Rech. Dept. of Pharmacol./Toxicol. and Neuroscience Program, Michigan State Univ., East Lansing, MI 48824.

In drug-naive subjects trained in a conflict procedure, the first doses of diazepam do not show the increase (or release) in punished responding observed with consequent doses. This "initial treatment phenomenon" seems not to depend on pharmacokinetic factors since this effect is seen whether diazepam is given daily or every 5 days. This may correlate with an initial depression in humans after benzodiazepines which tolerates out over two or three doses. The mechanism(s) by which this phenomenon occurs has not been investigated to any great extent. The purpose of the present experiment was to examine these mechanisms.

Female Sprague-Dawley rats (n=20) were water-deprived and trained to drink from a water tube in daily 10-min sessions. After drinking from the tube had stabilized a tone was present for 7 sec on a variable interval 21 sec schedule. During the last 5 sec of the tone drinking from the tube was accompanied by shocks. The number of shocks accepted and water intake were measured. Once behavior had stabilized, rats were divided into three groups. Group I received 3.0 mg/kg diazepam 10 min before and vehicle just after each test session for sessions 1-8; vehicle 10 min before and after the session in sessions 9-14; and 3.0 mg/kg diazepam 10 min before and vehicle after the session in sessions 15 and 16. Group II received vehicle 10 min before and 3.0 mg/kg diazepam after sessions 1-7; and 3.0 mg/kg diazepam 10 min before and vehicle after sessions 8-16. Group III received vehicle both before and after sessions 1-7; and 3.0 mg/kg diazepam 10 min before and vehicle after sessions 8-16. Diazepam was suspended in 0.5% methylcellulose. Drug and vehicle were injected i.p. every 3 or 4 days. Animals were run six days a week.

Group I showed the initial treatment phenomenon with the number of shocks increasing over the first three sessions. Group II and III did not show a significant change from baseline over the first seven sessions. In session 8 (first session that Groups II and III received diazepam pretreatment) Group II showed an increase in shocks which was significantly greater than Group I in session 1, while Group III in session 8 did not show an increase in punished responding significantly different from Group I-session 1. Group II showed an immediate plateau in shocks accepted while Group III showed a continuous increase until session 12. These data suggest that the initial treatment phenomenon relates in part to tolerance to non-specific depressant effects (not behavioral tolerance). Moreover, since Group II showed greater anticonflict activity after the first diazepam pretreatment, compared to Group I, a behavioral tolerance may have limited the peak anticonflict effect attained in Group I. Further evidence to this point as the gradual attrition in anticonflict effects of the drug with later repetitions in Groups II and III. (Supported by 3M Foundation and NRSA grant No. GM-07392).)

28.8 PENTOBARBITAL INHIBITION MEASURED INTRACELLULARLY IN HIPPOCAMPAL CA_3 NEURONS. M. O'Beirne*, N. Gurevich*, P.L. Carlen. Depts. of Physiology and Medicine, Institute of Medical Science, University of Toronto, Addiction Research Foundation Clinical Institute, Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, Canada.

It has previously been reported that barbiturates such as sodium pentobarbital exert their effects by increasing chloride conductance - an augmentation of γ-Aminobutyric acid response. Our data suggests that at lower doses a calcium-mediated potassium conductance may be involved.

Intracellular recording techniques were used to study the effects of sodium pentobarbital on the CA_3 hippocampal cell of the guinea pig. The drug was either perfused or applied by pressure ejection onto the hippocampal slices at concentrations of 10^{-6} or 10^{-5} M. One to 2 minutes after drop application or 3 to 5 minutes after drug perfusion, hyperpolarization of .2 to 8 mV in 5/7 cells occurred. Spontaneous activity when present decreased in 3/4 cells. In most cells the input resistance also decreased. The afterhyperpolarization (AHP) following a train of 2 to 4 spikes elicited by a 100 msec constant current depolarizing pulse was augmented in 4/6 cells. The two cells which did not show an augmentation of the AHP had been dramatically hyperpolarized following pentobarbital application. IPSPs were increased to a variable extent in 5/7 cells. The effects also occurred in 3 cells where K Cl electrodes were used suggesting that chloride channels may not be involved. In 2 CA1 cells, pentobarbital caused hyperpolarization and decreased spontaneous activity.

Alger and Nicoll (1980) have demonstrated that the AHP in CA1 cells is an intrinsic, calcium dependent potassium potential that is initiated by the preceding burst discharge.

These preliminary results suggest that one of the mechanisms of low dose sodium pentobarbital inhibition is by augmentation of calcium mediated potassium conductance similar to that found by Carlen et al using low dose ethanol (Science, Vol. 215, p. 306-309, 1982) and low dose benzodiazepine (Society for Neuroscience, Vol. 7, 1981).

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28.9 SYSTEMIC ADMINISTRATION OF PUTRESCINE OR MUSCIMOL CAUSES SIMILAR MODIFICATION OF AMPOMORPHINE-INDUCED BEHAVIOR IN RATS. F.D. Feng and B.B. Turner. Depts. of Psych. & Biol., Virginia Polytech. Institute & State Univ., Blacksburg, VA 24061.

Putrescine (PUT) is a diamine having several roles related to cellular metabolism and is also a minor precursor of GABA. We previously reported that oral and i.p. administration of PUT produced behavioral effects resembling those seen after treatment with GABA, GABA agonists, and GABA-transaminase inhibitors. Here we present data which suggest that systemic administration of PUT can modify apomorphine-induced (APO) stereotypic behavior in rats in a manner similar to that of the GABA agonist muscimol (MUS).

Male Sprague-Dawley rats (350-425g) were injected with saline (SAL), PUT (75 mg/kg) or MUS (.75 mg/kg). Fifteen minutes later subjects were given s.c. injections of either SAL or APO (.25 mg/kg). The incidence of locomotor activity and stereotypic behavior was time-sampled over a period of 45 min. Thirteen behavioral categories were scored; data were analysed by ANOVA statistics. Apomorphine per se caused stimulation of locomotor activity and stereotypic behaviors (non-body licking, mastication movements, and discontinuous sniffing). Treatment with MUS/SAL elicited a significant incidence of only mastication stereotypy; PUT/SAL treatment did not induce sterotypy. Neither MUS nor PUT in combination with SAL produced a change in locomotor activity.

Time-dependent drug interactions were found for the incidence of APO induced sniffing stereotypies following PUT or MUS pretreatment: discontinuous sniffing ($F(8,144)=2.49$, $p<.02$) and continuous sniffing ($F(8,144)=2.19$, $p<.05$). An apparent intensity increase, from discontinuous sniffing (DS) to continuous sniffing (CS), resulted from pretreatment with PUT or MUS; these effects lasted less than 60 min posttreatment. The SAL/APO group showed an increase in DS over the first 27 min of the time-sampling period; thereafter DS behavior decreased. The incidence of DS did not increase over time in either the MUS/APO or PUT/APO groups. At 19-27 min in the time-sampling, groups retreated with PUT or MUS were statistically similar and had a level of DS behavior which was significantly lower than that of the SAL group ($p<.05$). An increase in CS behavior was noted in subjects pretreated with PUT as compared with SAL at 10-18 min and 19-27 min of the observation period. During these periods subjects pretreated with MUS did not differ significantly from those pretreated with PUT.

These effects of PUT on APO-induced behavior are similar to those previously reported for MUS. Since PUT modified APO behavior in a manner similar to MUS, the actions of both agents may be expressed in part through common GABAergic mechanisms. (Supported by NIH grant 2-S07-RR-07095-13).

28.PO PRENATAL EXPOSURE TO DIAZEPAM RESULTS IN PERMANENT DEFICITS IN LEARNING AND REWARD-INDUCED EEG ALPHA-LIKE ACTIVITY IN CATS. G. T. Livezey*, L. Isaac, and T.J. Marczynski. Department of Pharmacology, Univ. of Illinois, Chicago, Ill., 60612.

There is much evidence that benzodiazepines act by enhancing GABAergic postsynaptic effects. The emergence of rhythmic synchronized EEG patterns of the alpha type is believed to depend on recurrent and/or feedforward inhibitory (hyperpolarizing) circuits involving GABAergic interneurons. Our previous study (Brain Res.,204:214,1981) showed that the magnitude and duration of the Postreinforcement EEG synchronization (PRS) over the visual cortex in cats trained to press a lever for 1 ml of milk reward are significantly correlated with learning ability .

In the present study we sought to determine whether or not there is a permanent effect of prenatal exposure to diazepam on learning ability, EEG PRS patterns, and the benzodiazepine receptor complement.

The pregnant cats, beginning on day 20 of gestation, were injected daily with diazepam according to the following schedule:

DAY	20 through 23	24-44	45-47	48-50	51-53	54-65
DOSE mg/Kg,i.m.	0.32	0.60	0.45	0.32	0.16	NO DRUG

The dose range did not exceed the human therapeutic doses. Control cats received saline injections. One year old offsprings were trained to press a lever for 1 ml of milk reward on a 1:7 fixed ratio schedule. The 20 min. daily training sessions consisted of standardized "shaping" procedure (Brain Res.204:214,1981) during which the experimenter challenged the animal's ability to eliminate perseveration in ineffectual approaches to the manipulanda. Following training, each cat was implanted with epidural cortical EEG electrodes, and the EEG patterns were monitored and quantified during several lever pressing sessions. Subsequently, the animals were sacrificed, and tissue samples were taken from three cortical areas, olfactory bulb, midline thalamus, caudate nucleus, hippocampus, hypothalamus, and ponse for ^3H Diazepam receptor binding tests.

Although the gross behavior, such as motor skills, playfulness, and eating habits were not apparently different from those of control animals, the drug exposed cats showed significant learning deficits (the time to achieve criterion of bar pressing was more than 5 times greater than that of control cats, and their 'perseveration index' was significantly higher), they showed a total loss of or poorly developed PRS responses (mean PRS index of 1.6 ± 0.2 versus 8.6 ± 1.1 S.D. of control cats), and a 40% reduction in binding of ^3H Diazepam to the fronto-orbital cortex and the hypothalamus. - The loss of PRS is consistent with the evidence that EEG 'spindles' contribute to plasticity of responses (Brain Res.235:51,1982).-Supported by Univ. of Ill. grant 58129.

29.1 DISTRIBUTION OF RADIOIMMUNOASSAYABLE ARGININE VASOPRESSIN (AVP) IN AREAS OF THE CEREBRAL CORTEX AND LIMBIC SYSTEM OF THE RAT BRAIN. M. Benke*, L. Mattiace* and A. Negro-Vilar, (Spon: Marcelle R. Morrison). Dept. of Physiology, Univ. of Texas Health Sci. Ctr., Dallas, TX 75235.

Recent studies on the behavioral effects of AVP and related peptides suggest a role for these compounds in memory consolidation processes. Indeed, the mnemonic process would require the participation of specific neuronal substrates in higher nervous centers. Although several reports have explored the extra-hypothalamic distribution of AVP, few studies have evaluated the presence of AVP in cerebral cortex. The present study was designed to evaluate the distribution of AVP in 15 areas of cerebral cortex as well as in several subcortical limbic structures of the rat brain, taking advantage of a highly specific and sensitive AVP RIA. Young adult male rats of the Holtzman strain were killed by decapitation, their brains removed, frozen on dry ice and 300μ-thick sections were serially cut. The following cortical and limbic structures were punched out: Frontal Polar Cortex (FPC), Pyriform Cortex (PFC, 5 sections, 1 through V rostral to caudal), Frontal Motor Cx (FMC) Cingular Cx (CGC) Entorhinal Cx (ERC, section I and II), Parietal Cx (PC) Insular Cx (IC) Hippocampus (HPC Sections I-III), Septum lateralis (SL), Stria terminalis (ST), Basolateral Amygdala (ABL) and Corticomedial Amygdala (ACO). Tissue punches were homogenized in acetic acid and appropriate aliquots assayed for AVP content, which was expressed per mg of protein. Most cortical areas explored had low but detectable levels of AVP, and could be divided according to AVP levels, in three groups: 1) areas with about 1 pmol AVP/mg protein (PFC-III, PFC-IV and CGC); 2) areas with values around 0.5 pmol/ mg prot. (HPC-I, II, PFC-I, II) and areas with very low (0.3 pmol/ mg prot.) to undetectable values (<0.05 pmol/mg prot.) such as FMC, ERC-I, IPC, PC, IC, PFC5 and ERC-II. Limbic areas had values at or above 1 pmol/mg protein, with the following rank order: ACO>SL>ST>ABL. These results indicate that small but readily detectable levels of AVP are distributed among many cortical areas. Regional differences within the same area also seem to be detected. The results on AVP levels in septum and amygdala confirm previous reports indicating the presence of AVP fibers in those regions. Taken together, our observations indicate that AVP is ubiquituously, albeit sparcely, distributed in many cortical and limbic regions, thus offering an anatomical substrate for the purported behavioral actions of this neural peptide. Supported by a grant from Smith-Kline and French.

29.2 LHRH CELLS: A VARIATION WITH MATURATION. S. Wray and G. E. Hoffman, Dept. of Anatomy, University of Rochester, School of Medicine and Dentistry, Rochester NY 14642.

This study immunocytochemically examined the LHRH system in both male and female rats from early postnatal life through to adulthood. All animals were perfused with a neutral picric acid formalin fixative. The brains were removed and postfixed in this same fixative for 2 - 12 hours. The tissue was then blocked and using a vibrating microtome, sections were cut, at 50 microns. Consecutive sections were taken from the level of the olfactory peduncles caudally through the median eminence. At least one male and one female rat were processed simultaneously to allow comparisons in staining intensities to be made.

The majority of LHRH cell bodies in all ages examined were present within a V-shaped field whose apex lay in the preoptic/ septal area and which extended caudally to the ventrolateral anterior hypothalamus. Although the regional distribution of LHRH did not vary with age in males and females, fluctuations were seen with age in total cell number visualized. For example, a decrease (by approximately one-half) in total cell number visualized occurred just prior to puberty in both sexes. This decrease most likely represents a physiological event rather than cell death due to the fact that total cell number returned to prepubertal levels in both male and female animals at later ages (approximately 1,200 cells).

In the adult, two LHRH cell types were characterized. Cell Type I is a bipolar cell with a large nucleus. Both its soma and dendrite are smooth in contour. Cell Type II is a smaller, irregular cell with many spine-like processes on its dendrite and/or soma. In the prepubertal animal, Type II Cells were present but a predominance of Cell Type I was found. Over development, a striking difference was seen in the ratio of Cell Type I to Cell Type II. In both males and females, the percent of Cell Type I decreased with age and, concomitantly, the percent of Cell Type II increased. The increase in cells with spine-like processes may be indicative of changes in afferent input to the LHRH system.

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29.3 RELATIONSHIP OF CATECHOLAMINES, NEUROTENSIN AND SUBSTANCE P TO LHRH CELLS. G. E. Hoffman, S. Wray, G. Pelletier and M. Goldstein. Dept. of Anatomy, University of Rochester School of Med. and Dent., Rochester, NY 14642, Med. Res. Council Grp. in Molec. Endocrinology, Centre Hospitalier de l'Universite, Laval, Quebec, Dept. Psychiatry, New York Medical Center, New York NY 10016.

A wealth of evidence suggests that catecholamines influence gonadotrophin secretion. Neuropeptides such as neurotensin (NT) and substance P (SP) are likewise implicated in the neuroendocrine control of reproduction. To assess whether direct interactions with these neuroeffectors and the LHRH cells were present, co-localization of tyrosine hydroxylase (TH), NT and SP were performed with either a dual immunoperoxidase or immunoperoxidase/ immunofluorescence technique. Adult rats and mice were perfused with a neutral picric acid formalin solution. The brain was removed and sectioned on a vibrating microtome at 20-25 microns. The sections were extensively rinsed in phosphate buffered saline before processing for dual immunocytochemical localization. Both methods produced satisfactory results. Initial experiments indicated that for optimal localization of LHRH and the other substances, the LHRH should be reacted first. No removal of the antibody complexes was necessary to achieve good separation.

A juxtaposition of catecholamine fibers on LHRH cells and their dendrites was noted. Not all the LHRH cells appeared contacted; the cell population close to the borders of the organum vasculosum of the lamina terminalis and adjacent preoptic area regions appeared favored. In addition, LHRH axons were in close apposition to the dopamine cells of the arcuate nucleus and periventricular hypothalamus. Within the median eminence, the anatomical distribution of LHRH and TH was differentially organized with few areas of overlap. These results support a direct action of catecholamines on the LHRH system and suggest that LHRH may influence dopamine function. This latter interaction may modify LH and FSH release through alterations in prolactin secretion.

Both neurotensin and substance P containing axons appeared to contact the LHRH cells. Like the patterns seen for catecholamines, these interactions did not appear to encompass all the LHRH cells and evidence for differential innervation was obtained.

In summary, all of the substances tested appeared to contact some of the LHRH cells. The fact that LHRH axons appeared to contact other LHRH cells may provide a means of integrating this diverse input.

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29.4 LHRH IMMUNOREACTIVITY AND SEXUAL DIMORPHISM WITHIN THE RAT PREOPTIC AREA. S. E. Hendricks, J. R. Leu, P. Lawson* and M. T. Hagley*. Dept. of Psychology, Univ. of Nebraska at Omaha, Omaha, NE 68182.

A nucleus within the medial preoptic area of the rat has been shown to exhibit a distinctive sexual dimorphism; being several times larger in males than in females (Gorski et al., Brain Res., 148:333, 1978). This Sexually Dimorphic Nucleus (SDN) appears as an intensely staining component of the medial preoptic area. We have attempted to provide a description of the juxtaposition of LHRH containing cells to this nucleus in rats. Male and female rats were perfused with saline followed by Zamboni's fixative and the brains post-fixed in Zamboni's for 48 hr. After 24 hr in 30% sucrose, 50 μ frozen sections were cut in frontal, horizontal, and sagittal planes. Alternate sections were stained with thionin for visualization of the SDN. The sections taken before and after those showing the SDN were immunocytochemically stained for LHRH using the unlabeled antibody (PAP) method of Sternberger. Some animals received daily injections of 100 μg estradiol benzoate for one week prior to sacrifice. Male and female brains were sectioned in each plane. The stained tissues were then described with special attention to the relationship between LHRH-containing cells and the SDN. The major LHRH pathways were not seen to pass through the SDN. However, a few LHRH immunoreactive fibers, but no cell bodies, were observed within and around the SDN.

29.5 CORTICOTROPIN RELEASING FACTOR (CRF): IMMUNOREACTIVE NEURONS AND FIBERS IN RAT AND PRIMATE HYPOTHALAMUS. E.L.F. Battenberg, Floyd E. Bloom, Jean Rivier, and Wylie Vale. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute, La Jolla, CA 92037.

Corticotropin Releasing Factor (CRF) was one of the first hypophysiotrophic factors to be detected but proved difficult to purify, isolate and characterize chemically. Vale et al., recently isolated, purified and synthetically replicated a 41 residue ovine hypothalamic peptide with potent secretogogic actions on cultured pituitary corticotropes. We report the immunocytochemical detection of CRF-immunoreactive (CRF-ir) neurons in rat and primate hypothalamus, as well as the nerve fibers arising from these neurons. Antisera were raised in rabbits against synthetic ovine CRF or an N-terminal fragment. A series of 12 sera were evaluated, in which the immunogen peptides either coupled to human serum albumin or polymerized, were conjugated to the haptene to emphasize exposure of the N-terminal, or remainder of the CRF sequence. Comparable immunocytochemical results were obtained with all antisera (dilutions 1;500-1;5000) regardless of the antigenic determinants of the immunogen. For immunocytochemical studies, rat and monkey brains were perfused with 4% paraformaldehyde, and processed for indirect immunoperoxidase localization as described by us. A heavy density of CRF-ir nerve fibers was observed in the median eminence. These fibers were found in the internal palisade zone in the anterior regions of the median eminence and moved to the external palisade zone as the tuberal region was approached. A few CRF-ir fibers were traced into the neural stalk. Within the rat hypothalamus, nerve fiber staining was seen in moderate density in the basal arcuate area near the location of corticotropin neurons. A thick thatch-work of fibers was found in the immediate sub-ependymal zone of the third ventricle. CRF-ir perikarya, only detectable in rats pre-treated with intracerebroventricular colchicine, were restricted to the rostral portion of the paraventricular nuclear complex. No positive perikarya were detected within the magnocellular regions of the paraventricular or supraoptic nuclei, although some CRF-ir fibers were detected in the latter nucleus in normal rats. In primate hypothalamus (squirrel monkey) and pituitary (squirrel monkey and human), a rich innervation of the median eminence was prominent, and posterior pituitary fibers were seen. Dispersed CRF-ir neurons were seen with no colchicine pre-treatment, located throughout the anterior periventricular area. These results strongly support the view that ovine CRF may play a major role in regulation of pituitary corticotropin secretion. Supported by USPHS DA 01785, AM 26741, and AA 03504.

29.6 CORTICOTROPIN RELEASING FACTOR (CRF) LOCALIZATION IN THE RAT BRAIN. S. Cummings*, R. Elde, J. Ells and A. Lindall. (SPON: G. Giesler). Univ. of Minnesota, Minneapolis, MN 55455 and ImmunoNuclear Corp., Stillwater, MN 55082.

A 41 amino acid peptide was recently characterized as CRF. To investigate the distribution of CRF throughout the brain, normal and colchicine treated rats were perfused with Zamboni fixative or 4% paraformaldehyde. Primary antiserum directed against CRF was used in both immunofluorescence and immunoperoxidase staining of cryostat and frozen sections, respectively. Specificity of staining was determined by absorption controls.

In the hypothalamus of both normal and colchicine treated rats CRF immunostained fibers were prominent in the external layer of the median eminence, while fewer fibers were found in the internal layer. CRF immunoreactive cell bodies were visible after colchicine treatment as intensely staining aggregations in parvocellular paraventricular nucleus and in the periventricular area, particularly at the caudal level of the optic chiasm. Numerous cell bodies were present throughout the medial preoptic nucleus. Scattered cell bodies were within the lateral and dorsomedial hypothalamic nuclei, premamillary nucleus, lateral mamillary nucleus and supraoptic nucleus as well as dorsal to the latter.

In the forebrain numerous CRF immunoreactive cell bodies were found in the lateral septal nucleus, nucleus accumbens, nucleus interstitialis stria terminalis and central nucleus of the amygdala. Fibers were present in the olfactory tubercle, in nucleus accumbens, stria terminalis and organum vasculosum lamina terminalis.

In the brainstem CRF cell bodies and fibers were present in raphe dorsalis, periaqueductal gray and perihypoglossal nucleus.

CRF immunoreactive neurons were predominately fusiform or multipolar. Immunoreactivity was limited to the cytoplasm of the cell body and proximal dendrites and varied in intensity according to the location of the neuron, suggesting variations in the rate of synthesis of CRF in different regions. Fibers were either coarsely beaded or of a fine diameter according to location.

Results of this investigation indicate a widespread occurrence of CRF immunoreactivity within the rat brain as well as within the hypothalamo-hypophyseal axis. The presence of CRF in neurons outside of the neuroendocrine axis implicates a possible role for this peptide in neurotransmission.

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29.7 LOCALIZATION OF CORTICOTROPIN RELEASING FACTOR-LIKE IMMUNOREACTIVITY AND RECEPTOR BINDING ACTIVITY IN OVINE BRAIN. J. Côté*, M. Lavoie*, P. Poyet*, F. Labrie* and N. Barden* (SPON: M. Beaulieu), MRC Group in Molecular Endocrinology, Le Centre Hospitalier de l'Université Laval, Quebec G1V 4G2, Canada.

Corticotropin-releasing factor (CRF) has recently been isolated from ovine hypothalamus and characterized as a 41-amino acid peptide. We have developed and used a sensitive and specific radioimmunoassay for this peptide to demonstrate the presence of CRF-like immunoreactivity in extra-hypothalamic areas of ovine brain. Rabbit anti-CRF serum was produced following repeated injection of synthetic CRF coupled to BSA in Freund's adjuvant. Anti-CRF serum was incubated with ^{125}I-labelled CRF and synthetic CRF standards or brain extracts for 24 h at 4° prior to separation of free and bound tracer by centrifugation after addition of goat-anti-rabbit serum and polyethylene glycol. Synthetic CRF displaced bound tracer at an ED_{50} value of 200 pg and there was no cross reactivity with LHRH, TRH, ACTH, β-endorphin and several other peptides. Discrete regions of ovine brain were homogenized in 20 vol of 2N acetic acid and following centrifugation, the supernatant was lyophylized prior to resuspension in assay buffer. Highest concentrations (3 ng/mg tissue) of CRF-like immunoreactivity were found in the median eminence but appreciable amounts were also noted in hypothalamus, thalamus, amygdala, substantia nigra, septum, habenula and cerebral cortex. Lower concentrations were noted in other brain areas, including the basal ganglia and brain stem. Displacement of bound ^{125}I CRF by brain extracts exhibited curves parallel to synthetic CRF standards. ^{125}I CRF specifically bound to cell membranes prepared from several brain areas, including thalamus, basal ganglia and substantia nigra, but not to membranes prepared from cerebral cortex. Although we do not as yet know either the molecular nature of CRF-like immunoreactivity or its functions, these results indicate, for the first time, the presence of CRF or related molecules and receptor binding activity in widely different regions of ovine brain.

29.8 LOCALIZATION AND CHARACTERIZATION OF THYROTOPIN-RELEASING HORMONE IN THE MEDULLA OBLONGATA OF THE RAT. M.A. Rea, M.J. Kubek, Z.I. Hodes* and M.H. Aprison. Depts. of Psychiatry, Anatomy and Biochemistry, Indiana Univ. Sch. of Medicine, Indianapolis, IN 46223

TRH has been localized throughout the CNS of man and several animal species. Evidence suggests that TRH may act as a neurotransmitter or neuromodulator in the CNS. TRH has been shown also to elicit several neuropharmacological effects. Since some of these effects can be associated with vagal complex functions, we sought to quantitatively measure as well as characterize TRH in specific loci of the caudal medulla oblongata of the rat. Regions containing the dorsal motor nucleus of the vagus (DMN), nucleus tractus solitarius (NTS), hypoglossal nucleus, dorsal column nuclei, descending nucleus V (DNV), nucleus ambiguus (NA), raphe nuclei, dorsomedial and ventromedial reticular formation, and inferior olivary nuclei, were isolated from six 300μm thick frozen sections of medulla (from 900μm caudal through 900μm rostral to the obex) using micropunches constructed of 18 and 20 gauge stainless steel needles. Each region was pooled bilaterally, homogenized in 0.1 N HCl, and vacuum dried. Extracts were assayed for TRH by specific radioimmunoassay (RIA). TRH levels varied 100-fold among medullary nuclei. Highest content (ng/mg protein±SD) were found in DMN (14±4) and NTS (5±2), while lowest levels occurred in the DNV (0.13±0.06). Nearly 65% of the total medullary TRH was localized to nuclei associated with vagal complex (DMN, NTS, NA). Characterization of tissue immunoreactivity (TRHi) in these regions suggests the presence of TRH since: (1) medullary tissue extracts competed with ^{125}I-TRH for antibody binding sites with the same affinity as authentic TRH: (2) TRHi in tissue extracts comigrated with synthetic TRH when subjected to reversed phase high performance liquid chromatography and Sephadex G-10 chromatography; and (3) rat serum TRH peptidases degraded TRHi and authentic TRH at similar rates. Another group of rats were subjected to unilateral (right side) vagotomy. At 33 weeks post-vagotomy, the vagal preganglionic cell population in the ipsilateral DMN was depleted 50-75% while the contralateral side was unaffected. Interestingly, the content of TRH in the ipsilateral (right) DMN remained unchanged (14±4) whereas TRH in the contralateral DMN increased by 50% (21±4; $P<0.05$). The content of TRH in both ipsi- and contralateral NTS was unchanged when compared to sham-operated controls. These results indicate: (1) TRH is present in several specific loci of the medulla, (2) very high levels are found in the vagal complex, and (3) vagotomy may alter TRH in the contralateral DMN. Further studies are in progress in our laboratories to identify the source(s) and function of vagal TRH. (Supported in part by NIH research grants RO1-NS 16205 (MHA) and RO1-AM 28260 (MJK).)

29.9 **TRH IN THE LAMPREY CNS.** L.J. Youngs*, A. Winokur, N.R. Krieger and M.E. Selzer (SPON: D.S. Silberberg). Departments of Neurology, Pharmacology and Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

TRH levels were measured in brains and spinal cords of larval and young adult sea lampreys (P. marinus) and adult river lampreys (I. unicuspis) by radioimmunoassay (RIA). The entire CNS was removed under Tricaine anesthesia. Brains and lengths of spinal cord were weighed, homogenized in PBS pH 7.5, then combined with methanol, and centrifuged at 2,000 RPM. The supernatant was air dried, reconstituted in BSA/PBS, and assayed for TRH content by the RIA procedure of Bassiri and Utiger (Endocrinol. (1972)90: 722). The identity of immunoreactive TRH with authentic TRH was confirmed by HPLC.

In larval P. marinus, brain TRH was 94.2 pg/mg wet wt. ∓21.6 SEM (n=7). Larval cord values were 12.5∓2.4 (n=10). Young adult sea lamprey brain levels were 85.5 pg/mg ∓20.7 (n=5) and mean cord levels were 10.1 (n=2). For adult river lampreys brain TRH was 137.5 pg/mg (n=2) and cord TRH was 10.9 (n=2).

We conclude that: 1) the concentration of TRH in the brain is about 8-12 times that of the spinal cord; 2) there is little difference in CNS TRH levels between large larvae and adult forms; 3) TRH levels are similar in the two genuses studied.

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29.10 IMMUNOHISTOCHEMICAL LOCALIZATION OF THYROTROPIN-RELEASING HORMONE (TRH) IN THE RAT BRAIN. R. M. Lechan* and I. M. D. Jackson* (Sponsor: S. Reichlin). Endocrine Division, Tufts-New England Medical Center, Boston, MA 02111.

The perikaryal and fiber distribution of immunoreactive TRH in the rat CNS was demonstrated using the peroxidase-antiperoxidase (PAP) technique after rapid fixation with acrolein.

Six Sprague-Dawley rats (bw 250-350 g) were perfused through the ascending aorta with 5% acrolein (Polyscience Inc.) in a 0.1M phosphate buffer, pH 7.2 for five minutes. Three animals were pretreated with 75 µg of colchicine, stereotaxically injected into the lateral ventricle two days prior to killing. 60 µm sections of the forebrain, brain stem and spinal cord were made on a vibratome and incubated with a well characterized antiserum to TRH at a final dilution of 1:750 containing 0.3% Triton X-100. The reaction product was developed with DAB.

In colchicine treated animals, immunoreactive neuronal cell bodies were found predominantly in the hypothalamus, especially in the parvocellular subdivison of the paraventricular nucleus, suprachiasmatic subdivision of the preoptic nucleus and dorsomedial nucleus. Cells were also present in the posterior magnocellular part of the PVN, perifornical region, lateral hypothalamus, arcuate nucleus, region of the prelateral mammillary nucleus, bed nucleus of the stria terminalis, medial preoptic area and in the diagonal band of Broca. Only rare cells were present in the corticomedial nucleus of the amygdala. In the brain stem, numerous cells were identified in the raphe magnus, pallidus and obscuris. No immunoreactive perikarya were present in the spinal cord.

Immunoreactive neuronal processes were identified in nearly all regions of the hypothalamus but predominantly in the external zone of the median eminence extending into the posterior pituitary, OVLT, parvocellular subdivision of the paraventricular nucleus, periventricular nucleus, perifornical region, dorsomedial nucleus and arcuate nucleus. In other regions of the forebrain, a high density of terminal fields was present in the lateral septal nucleus, bed nucleus of the stria terminalis, corticomedial nucleus of the amygdala, zona incerta and periventricular nucleus of the thalamus. Reaction product was also seen in many regions of the brain stem and was particularly rich in the parabrachial nucleus, trigeminal nucleus, nucleus tractus solitarius, nucleus commissuralis and nucleus intercalatus. In the spinal cord, immunoperoxidase was present in the central grey, intermediolateral cell column and about α-motoneurons in the ventral horn.

These studies demonstrate a wide distribution of TRH throughout many different anatomic regions of the CNS and suggest other roles for TRH as a neurotransmitter and/or neuromodulator independent of its action as hypophysiotropic hormone.

29.11

WITHDRAWN

29.12 IMMUNOCYOCHEMICAL LOCALIZATION OF HISTIDYL-PROLINE DIKETOPIPERAZINE IN INTACT PITUITARY AND PITUITARY MONOLAYER CULTURES. V. May, G. V. Childs* and J. F. Wilber*. Northwestern Univ. Med. Sch., Chicago, IL 60611, Univ. of Texas Med. Branch, Galveston, TX 77550, Louisiana State Univ. Med. Sch., New Orleans, LA 70112

Thyrotropin-releasing hormone (TRH) has been localized immunocytochemically in the secretory granules of pituitary cells in both the intact rat anterior pituitary (Childs et al., J. Histochem. Cytochem. 26:901, 1978) and in pituitary monolayer culture (May et al., J. Histochem. Cytochem. 29:900, 1981). Histidyl-proline diketopiperazine (cyclo his-pro), a cyclic dipeptide formed by pyroglutamate aminopeptidase proteolysis of TRH, has been shown to be biologically active in the central nervous system. Specific antiserum against cyclo his-pro has been developed for use in RIA by Mori et al. (Endocrinol. 108:1955, 1981) that exhibits less than 0.12% crossreactivity with TRH. We now describe its application in immunocytochemical staining of intact pituitary and cell cultures. For intact tissue studies, adult male rat pituitaries were fixed in 1% glutaraldehyde and embedded in Araldite 6005. To determine whether cyclo his-pro could also be found in cells free of external influences, anterior pituitaries were dispersed and grown as monolayer cell cultures for 7-21 days. The cell pellets were then fixed and embedded. Ultrathin sections of intact pituitaries and cultured pituitary cells were stained using anti-cyclo his-pro with the peroxidase anti-peroxidase (PAP) complex technique. A titration curve gave an antiserum working dilution of 1:25,000. Cyclo his-pro immunoreactivity in the intact pituitary was localized in secretory granules with varying intensity in cells that resembled thyrotropes, gonadotropes and corticotropes. An intense staining reaction persisted in pituitary cells maintained in culture which was also localized to the secretory granules. Liquid phase absorptions with 10ng-100ng/ml cyclo his-pro attenuated the staining intensity to below control values. Absorption specificity tests using 1µg/ml LH, FSH, ACTH, TSH, GnRH and TRH failed to alter staining intensity. Solid phase immunoabsorption tests to further demonstrate the specificity and experiments with serially sectioned cells stained for cyclo his-pro, TRH, ACTH, TSH and the gonadotropins for positive identification of the stained cells are presently in progress. These studies demonstrate that the PAP complex technique is capable of detecting cyclo his-pro immunoreactivity in fixed and embedded pituitary tissue and that the cyclo his-pro immunoreactivity persists in pituitary cell cultures. The staining pattern of the cyclo his-pro antiserum is very similar to that for TRH immunoreactivity to suggest a parallelism in their sites of localization. Supported by PHS 5-T32-H07068 (JM) and NIH AM25482 (JFW).

29.13 **IMMUNOCYTOCHEMICAL LOCALIZATION OF TRH IN RAT HYPOTHALAMUS.**

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The distribution of TRH in the vertebrate CNS has been extensively described by radioimmunological procedures. However, there have been few reports of its immunocytochemical localization. Hökfelt et al (Eur. J. Pharm., 34: 389, 1975) and Choy and Watkins (Cell Tiss. Res., 177: 371, 1977) used indirect immunofluorescence and PAP immunoperoxidase techniques to investigate the in situ localization of TRH in the hypothalamus. We have employed a different immunocytochemical method which utilizes avidin-biotin complexed horseradish peroxidase to examine the distribution of TRH immunoreactive perikarya and processes in the rat hypothalamus.

Male rats (150-200 g) were perfused with phosphate buffered saline followed by lysine periodate fixative 24 hours after pretreatment with colchicine (60 - 120 µg/50 µl 0.9% saline, intracisternal). 50 µ cryostat sections were processed for immunocytochemistry by the ABC method (Vector Laboratories). The sections were incubated with TRH antisera (1:500) overnight at 4° C. The antisera were raised in rabbits against TRH-BDB-BSA as described by Bassiri and Utiger (Endocrinol., 90: 722, 1972). Antisera which had been incubated with 1 X 10^{-4} M TRH-BDB-BSA overnight served as the control sera.

Numerous immunoreactive cell bodies were observed in the periventricular and dorsomedial nuclei of the hypothalamus. A few positive cell bodies were seen in the paraventricular nucleus. The external layer of the median eminence contained the densest network of immunoreactive fibers. The dorsomedial nucleus contained a moderate density of positive fibers. Other hypothalamic areas contained fewer immunoreactive processes. The staining was eliminated by preabsorption of the antiserum with TRH-BDB-BSA but not after incubation with BDB-BSA.

These findings provide further evidence for the cellular localization of TRH in nuclei in the rat hypothalamus. The utility of the avidin-biotin immunoperoxidase method for studies of the cellular localization of TRH in other areas of the CNS is under investigation in our laboratory.

29.14 SOMATOSTATIN AND THE ANTERIOR OLFACTORY NUCLEUS OF THE MOUSE.

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The anterior olfactory nucleus (AON), a relay structure of the olfactory system, provides major afferents to the main olfactory bulb (OB). Although somatostatin (SS) cells are present in discrete areas of the AON and scattered throughout the OB, no attempts have been made to examine the axonal distribution of these neurons. The present study was designed to assess the localization of SS in olfactory structures of the murine brain.

Adult male mice were perfused intracardially with Zamboni's fixative and brain sections were cut at 30 microns on a vibrating microtome. The sections were processed using the unlabeled antibody enzyme method or with immunofluorescence techniques. SS cells were found scattered in the rostral extent of AON in pars lateralis and externa. A more dense population of cells surrounded the anterior commissure at the interface with AON, as well as in the pyriform cortex and olfactory tubercle. Sparse fiber networks were observed around AON, and in the external plexiform layer, granule cell layer, and medial periglomerular surfaces of the main OB. A moderately dense SS fiber plexus was found in the superficial layers of taenia tecti and caudal aspects of the olfactory tubercle. Axons were also seen in the superficial layer of prepyriform cortex coursing towards the rhinal fissure, where they ended abruptly.

Because SS fibers are present in OB, we sought to determine if these fibers represented axons of the AON SS neuronal pool. Using immunoperoxidase or immunofluorescence in combination with retrograde tracers injected into OB, we found that less than 3% of SS labeled cells in AON were retrogradely labeled. These results suggest that the SS neurons of AON give rise to axons that are distributed to areas other than OB, perhaps to contralateral AON or prepyriform cortex and that AON represents a heterogeneous neuronal population with a diverse projection field.

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29.15 DESCENDING HYPOTHALAMIC OXYTOCIN/MESOTOCIN PROJECTIONS END PREFERENTIALLY ON PULMONARY AND BARORECEPTOR REGIONS OF THE N. SOLITARIUS.

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Vagal sensory ganglion neurons innervate cardiovascular, gastrointestinal and pulmonary structures. Their central projections terminate in organotopically specific subnuclei of the nucleus of the Tractus Solitarius (nTS; Katz and Karten '77,'78,'79,'82). The identification of putative neurotransmitters used by vagal sensory afferents, as well as those from central systems which may modify incoming information, is a matter of considerable interest. We have begun investigations of the organization of nTS in the pigeon with antisera directed against a number of peptide and non-peptide antigens, including oxytocin and neurophysin.

There is a specific distribution of oxytocin-like imunoreactivity (OLI) in the nTS. A region of dense immunoreactive terminals is found predominately within the nucleus sulcalis dorsalis and in the nucleus parasolitarius lateralis (Katz and Karten, in press). These subnuclei receive their vagal inputs from baroreceptor and pulmonary targets, respectively (Katz and Karten, 1979). The pattern of neurophysin-like immunoreactivity (NLI) is identical to that of OLI. A sparse scattering of OLI- and NLI-containing axons were found surrounding the entire nTS complex. The neurophysin antiserum consisted of the IgG fraction of an antiserum which recognizes all three neurophysins. Preincubation of the oxytocin antiserum with synthetic peptides (10µM) indicated no cross-reactivity at the immunohistochemical level with arginine vasopressin, arginine vasotocin (the avian equivalent of arginine vasopressin) or the neurophysins. Preincubation of the oxytocin antiserum with mesotocin (1µM), the avian equivalent of oxytocin, abolished staining as did 1µM oxytocin controls.

Various pathway tracing techniques have shown that the medial preoptic area, the anteromedial hypothalamus and the nucleus periventricularis magnocellularis (PVM) project to brainstem (Finkelstein and Berk, 1980; Berk and Butler, 1981). Furthermore, neurons in these areas exhibit oxytocin-like, vasotocin-like and neurophysin-like immunoreactivity. We have, therefore, begun studies to determine which diencephalic nuclei give rise to the projection to nTS. Preliminary results from our HRP injections indicate that, among other areas, a restricted subdivision of the PVM may be a source of the OLI- and NLI-containing projection to the nTS. Autoradiographic studies of descending projections of PVM are consistent with these results although the exact trajectory of this pathway remains to be clarified. Supported by NS12078 to H.J.K. and HL24103 to J.B.C.

29.16 **COMPARISON BETWEEN NEURONS DEMONSTRATING SOMATOSTATIN-LIKE IMMUNOREACTIVITY AND THOSE CONTAINING ACETYLCHOLINESTERASE IN THE RAT FOREBRAIN**

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In order to demonstrate whether or not somatostatin-like immunoreactive (SLI) neurons of the forebrain also stained for acetylcholinesterase (AChE, E.C. 3.1.1.7), we processed brain tissue for fluorescent immunohistochemistry, and, following microscopic examination, counterstained the same tissue sections for AChE according to the pharmacohistochemical regimen (Butcher, L.L., & Bilezikjian, L., Eur. J. Pharmacol. 34:115, 1975).

SLI neurons were found in the frontal, cingulate, parietal, insular, and pyriform cortices, but not in the limbic cortex. Most cortical cells were weakly reactive for AChE, and no SLI cells in the cortex contained appreciable amounts of AChE. Other SLI cells were found in the caudate-putamen complex, nucleus accumbens, olfactory tubercle, nucleus of the diagonal band, lateral preoptic area, anterior amygdalar area, periventricular hypothalamus, and the central, lateral, and basolateral nuclei of the amygdala. Although intensely AChE-stained neurons were found in the caudate-putamen, nucleus accumbens, olfactory tubercle, nucleus of the diagonal band, and the lateral preoptic area, and moderately to intensely AChE-stained neurons were found in the periventricular hypothalamus, the more intensely AChE-stained cell population did not overlap with the SLI neuronal population.

In the caudate-putamen the SLI neurons had maximal soma extents ranging 14-25 µm, whereas intensely AChE-staining neurons in the caudate-putamen measured 20-44 µm. The SLI cells of the striatum had the following distribution of soma shapes: oval, 49%; fusiform, 36%; triangular, 9%; and round, 6%.

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29.17 SOMATOSTATIN-IMMUNOREACTIVITY IN THE BRAINSTEM: A COMPARISON OF METHODS. B. J. Morley and J. I. Koenig*, Res. Div., Boys Town Inst., Omaha, NE 68131 and Dept. Physiology, Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

Somatotrophin release inhibiting factor (somatostatin; SRIF) has been demonstrated in several non-hypothalamic areas of rat brain, including the brainstem (Krisch, B., Cell Tiss. Res., 217: 531, 1981; Takatsuki et al., Brain Res., 213: 211, 1981; Finley et al., Neurosci., 6: 2173, 1981). There are discrepancies among these reports with respect to the appearance of SRIF-immunoreactivity in adult animals and whether immunoreactivity is found in both cell bodies and terminals, or only in terminals. Since the concentration of somatostatin in the brainstem is lower than in hypothalamic areas, its visualization is likely to be more easily affected by variations in procedure.

We have systematically investigated several variables which might affect SRIF-immunoreactivity, including the source of the primary antibody, type of fixation, method of sectioning, colchicine-treatment, and the addition of agents promoting the penetration of antibodies into the tissue. We also compared the unlabeled peroxidase (PAP) and the avidin-biotin complex procedures.

Most of these variables affected the intensity of SRIF staining and some combinations of these variables resulted in the absence of staining. In our hands, the most significant single variable was penetration of the primary antibody. By varying fixation and optimizing penetration, we obtained heavy SRIF staining in both cell bodies and terminals, even without the use of colchicine treatment or pronase. Under optimal conditions we have noted no difference in the distribution of anti-somatostatin prepared by us and commercially available anti-somatostatin.

In comparison with previous research, our results are similar to those recently reported by Finley et al. (Neurosci., 6: 2173, 1981), in which pronase treatment was used to enhance the labeling of SRIF-immunoreactivity. Our results confirm the presence of SRIF-immunoreactivity in the brainstem and also suggest that the absence of SRIF-immunocytochemical labeling must be interpreted cautiously when more than one procedure is not investigated. (This research was supported in part by Biomedical Research Development Grant 1-S08-RR09003).

29.18 EFFECTS OF CYSTEAMINE ON BRAIN SOMATOSTATIN-14, SOMATOSTATIN-28 LI AND SOMATOSTATIN-28(1-12) LI. C. Bakhit, R. Benoit, and F.E. Bloom. The Salk Institute, La Jolla, CA 92037.

Three peptidic fragments related to somatostatin have been identified in rat brain. The original peptide somatostatin-14 (SS14) corresponds to the C-terminus of somatostatin-28 (SS28) whose N-terminus is somatostatin-28(1-12) [SS28(1-12)]. We have recently characterized the distribution of these somatostatin related peptides in selected rat brain regions using three specific radioimmunoassays: antiserum S310 recognizes only SS14, antiserum S329 reads the middle region of SS28 but not SS14 nor SS28(1-12) and antiserum S320 reads SS28 (1-12) but not SS14 and not SS28. The highest tissue concentration of the three peptides was found in hypothalamus and lowest in cerebellum. SS28(1-12)-like immunoreactivity (LI) was either equal to or more abundant than SS14. [Benoit et al. Endocrinol. 110, Abs. #913 1982).]

We report here the effects of cysteamine on the levels of the three somatostatin related peptides in selected regions of rat brain. Male Sprague-Dawley 200 g rats were injected intracerebroventricularly (ICV) with a 20 µl solution containing either saline or cysteamine. Rats were killed 2 1/2 hours later and the following brain regions dissected: cerebral cortex (CC), hippocampus (H), neostriatum (NS), hypothalamus (HY), and pons-medulla (PM). Tissues were collected, boiled, and extracted with 2M acetic acid and the extract analyzed by RIA.

Cysteamine (1400 µg/rat) produced a marked depletion of SS14 in all regions tested (% depletion: CC 37, H 48, NS 31, HY 45, PM 37). SS 28 LI was not significantly altered in all regions except H where there was a 22% depression. SS28(1-12) LI was not significantly altered in all regions except PM in which a 21% depression was observed. The above ICV dose of cysteamine produced in rats various behaviors which included: running fits, tonic-clonic seizures, barrel rotation, piloerection and frozen stares. A lower ICV dose of cysteamine (500 µg/rat) also produced a marked depletion of SS14 but did not alter the other two peptides. None of the behavioral effects described above were observed with the 500 µg ICV dose of cysteamine. Subcutaneous injection of cysteamine (90 mg/kg) also produced a selective depletion of SS14 but no obvious behavioral effects. The decrease of SS14 content was in agreement with recent studies by Sagar, S.M. et al. J. Neurosci. 2: 325, 1982 and Szabo, S. and Reichlin, S. Endocrinol. 109: 2255, 1981.

This study demonstrates that cysteamine selectively depletes SS14 in all brain regions tested and only marginally affects SS28 LI and SS28(1-12) LI in certain regions. This research is supported by NIAAA 07273 and AM 26741.

29.19 CHOLECYSTOKININ: RADIOIMMUNOASSAY, IMMUNOHISTOCHEMISTRY, RECEPTOR BINDING AND PHYSIOLOGICAL ACTIONS IN SPINAL CORD CULTURES. S.E. Hays*, M.A. Rogawski, M.C. Beinfeld*, L.R. Skirboll, T. Hökfelt* and G. Norell*. SRI International, Menlo Park, CA 94025; Lab. of Neurophysiology, NINCDS, Bethesda, MD 20205; Dept. of Pharmacology, St. Louis Univ. Med. Sch., St. Louis, MO 63104; Neuroscience Branch and Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

The spinal cord is richly innervated by cholecystokinin (CCK)-containing fibers. Although a major fraction of these may be derived from sensory ganglia and supraspinal descending systems, recent studies have demonstrated the existence of CCK-immunoreactive neuronal perikaria within the spinal cord. In this report, we give evidence that CCK-containing neurons are maintained in dissociated culture and present physiological data suggesting their functional role.

Spinal cords with some adherent sensory ganglia were cultured from 12 to 14 day old C57BL/6 mouse embryos. Following 4 to 6 weeks in vitro, the cells were harvested and extracted with methanol for radioimmunoassay (Beinfeld et al., Brain Res. 212: 51, 1981). The cultures contained levels of immunoreactive CCK-8 comparable to that found in adult spinal cord (0.97 ± 0.23 ng/mg protein). In contrast, pure cultures of dissociated spinal ganglia demonstrated less immunoreactivity (0.24 ± 0.03 ng/mg protein). Immunofluorescence staining of spinal cord cultures grown on glass coverslips revealed occasional islands containing dense networks of immunoreactive fibers with numerous brightly fluorescent varicosities that formed rosettes around the soma and processes of spinal cord neurons.

To determine if receptor sites for CCK are present on cultured neurons, radioligand binding was carried out using [^{125}I]CCK-33 (0.4 nM). Crude synaptosomal membranes prepared from frozen tissue demonstrated specific high affinity binding sites similar to those found in brain membranes. Binding was displaced by CCK-8 with an IC_{50} of 5 nM whereas desulfated CCK-8 had five-fold lower affinity for the CCK receptor. The functional role of CCK-8 was explored by applying the peptide to spinal neurons during intracellular recording. In a subpopulation of cells, CCK-8 (0.2-100 µM) produced a long lasting (20-200 sec) enhancement of action potential generation in response to stimulation with intracellular depolarizing current while desulfated CCK-8 had little or no such activity. In many cases, these effects on excitability were accompanied by a small membrane depolarization (0.5-4 mV) and a decrease in membrane conductance (5-19%). These studies demonstrate that spinal CCK-containing neurons can survive in cell culture. The existence of functionally active CCK-receptors on some spinal neurons supports the idea that the peptide serves as a neurotransmitter whose function may be to tonically modulate the excitability of target neurons within the cord.

30.1 REGIONAL DISTRIBUTION OF CATECHOLAMINES IN THE BRAINSTEM AND SPINAL CORD OF THE DOG. K.B. Brosnihan, R.C. Speth, P. Bridle*, C.L. Chernicky and C.M. Ferrario. Cleveland Clinic Research Division, Cleveland, OH 44106.

Little work has been done on the distribution of catecholamines in the dog's brainstem. The localization of catecholaminergic cell bodies and varicosities in the medulla of the dog was was first determined in preliminary studies with glyoxylic acid-induced fluorescence (unpublished results); the localization of the locus coerulus (LC) and A5 nucleus in the pontine tegmentum of the dog was based upon the study of Ishikawa et al. (Brain Res. 86: 1, 1975). Six male adult dogs (10-20 kg) were anesthetized with sodium pentobarbital. The brains were removed within 4 minutes of administration of an overdose of pentobarbital and were frozen immediately on dry ice. A block of brainstem tissue beginning 6 mm caudal to the obex and terminating at the level of the inferior colliculus was sliced in 2 mm sections. The spinal cord was removed from C7 to T3. Micropunches (13 or 15 ga) from the regions of the lateral reticular nucleus (A1), the dorsal motor nucleus of the vagus and nucleus tractus solitarius (A2), A5, LC, area postrema (AP), hypoglossal nucleus (HN), pyramidal tract (PT) and intermediolateral cell column (IML) were assayed for catecholamines radioenzymatically, and values expressed per mg protein. Norepinephrine (NE), epinephrine (EPI) and dopamine (DA) were measured on combined punches from these regions. Following dissection of the nuclei the remaining tissue in each slice was assayed for catecholamines.

	NE	EPI	DA
LC	19.76 ± 3.96	1.05 ± 0.14	1.63 ± 0.25
A2	14.60 ± 1.96	1.03 ± 0.15	1.48 ± 0.24
HN	10.10 ± 2.05	0.50 ± 0.13	0.77 ± 0.16
A5	8.56 ± 0.86	0.61 ± 0.11	0.60 ± 0.08
IML	8.04 ± 1.56	0.13 ± 0.04	0.39 ± 0.10
A1	6.36 ± 1.03	0.31 ± 0.03	0.50 ± 0.08
AP	5.32 ± 1.26	0.70 ± 0.22	1.33 ± 0.25
PT	1.30 ± 0.40	0.08 ± 0.02	0.14 ± 0.04

$\bar{X}$ ± S.E. (ng/mg protein) (n = 6)

Highest levels of NE were found in the LC and A2 regions. All regions punched except the PT possessed substantially higher levels of catecholamines than found in remaining tissue. The results indicate that the distribution of catecholamines in the dog brainstem and spinal cord is similar to that reported for other species. (Supported by an NHLBI grant [HL-6835]).

30.2 ESTIMATION OF DOPAMINE TURNOVER IN STRIATAL, LIMBIC AND FRONTAL CORTICAL AREAS OF HUMAN BRAIN. P.J. Langlais* F.X. Walsh* T.J. Stevens* and E.D. Bird*. (SPON: A. Pope). Brain Tissue Resource Center, McLean Hosp., Belmont, MA 02178

Recent studies in rat brain have shown that the rates of dopamine (DA) synthesis and release are several fold greater in prefrontal cortex than in striatum, limbic areas or olfactory tubercle (Bannon et al., Brain Res. 218:376-382, 1981). This same mesocortical DA projection system has received considerable attention since its response to chronic neuroleptic treatment also differs from that observed in other DA systems (Bacopoulos, N.G. et al., Brain Res., 157:396-401, 1978) and has been implicated in behavioral disturbances both in animals and in man. In the present study, the levels of DA and homovanillic acid (HVA), the major DA metabolite in human brain, were measured in the caudate, n. accumbens, frontal cortex (Brodmann A10), cingulate gyrus (A24) and paraolfactory gyrus (A25) of human post-mortem brain. Brain tissue was obtained and stored at -70°C from 12 patients who were free from neurological disease and any psychoactive drug therapy. The concentrations of DA and HVA, determined by reversed-phase, high performance liquid chromatography coupled with electrochemical detection, were found to be much higher in the caudate and accumbens than in any of the three frontal areas. The concentration of DA in two of the frontal cortical areas, A10 (.0025 ng/mg) and A24 (.005 ng/mg) were quite similar but was significantly higher in A25 (.680 ng/mg, $p<.001$). The concentration of HVA was also significantly higher in A25 (1.949 ng/mg, $p<.001$) than in A10 (.133 ng/mg) or A24 (.254 ng/mg). When the turnover of DA was estimated by calculating HVA/DA ratios, DA turnover was found to be significantly higher in A10 (86.1, $p<.001$), A24 (73.2, $p<.001$) and A25 (5.9, $p<.01$) when compared to caudate (1.8). Interestingly, the turnover of DA in A25 was significantly different from that of A10 and A24 but not significantly different from that of n. accumbens. These findings suggest that the synthesis and release of DA within terminals innervating frontal cortex differ significantly from striatal and limbic structures of human brain. These findings may indicate that frontal DA terminals may lack autoreceptors as has been proposed in rat frontal cortex.

30.3 ASYMMETRIES IN HUMAN BRAIN DOPAMINE RECEPTOR BINDING: RELATIONSHIP TO MIDBRAIN DOPAMINE CELL NUMBER. D.C. German, D.S. Schlusselberg*, B.A. McMillen, K. McDermott*, W.K. Smith*, and D.J. Woodward. Depts. of Physiol., Psychiat., Cell Biol., and Pharmacol., Univ. of Texas Health Sci. Cntr, Dallas, TX. 75235.

Midbrain dopamine (DA)-containing neurons decrease in number with aging and are abnormally sparse in Parkinson's disease. In mice, there is a positive correlation between the number of nigral DA neurons and the number of striatal ^{3}H-spiperone binding sites (putative DA D2 receptor sites). The present experiment was undertaken to determine whether the number of midbrain DA neurons is correlated with the number of ^{3}H-spiperone binding sites in the human brain.

Fresh human brains were obtained 12-14 hours postmortem. The left and right nucleus accumbens (NA), caudate nucleus (CAU), and putamen (PUT) were dissected out. For each brain area we measured the receptor number and affinity for ^{3}H-spiperone binding (Scatchard analysis), the level of DA, and its metabolite, DOPAC. The most obvious differences in the six brains examined (17-51 years of age) was a 21-26% difference in the number of binding sites on the two sides of the brain: for the NA, 3 brains were right predominant and 2 were left predominant; for the CAU 2 were right predominant; and for the PUT 5 brains were right predominant. In one brain there was a marked and general right predominance such that there were more binding sites on the right NA (44%), CAU (37%) and PUT (48%) vs. the left.

A system built around a Data General Eclipse (S/130) minicomputer and Ikonas Color Graphics system was used to map the location of the midbrain DA neurons. Cells containing neuromelanin pigment in 50 µm thick Nissl stained sections were identified as catecholamine-containing neurons. Sections were analyzed from the rostral pons to the level of the caudal mammillary bodies (12-14 mm). This system allows one to count and reconstruct the 3-dimensional topography of the midbrain DA neurons. In 3 brains reconstructed so far, there were differences in the number of DA cells on the two sides of the brain.

The anatomical reconstruction technique will allow examination of laterality effects, aging effects, areas of cell loss relative to specific parkinsonian symptomatology, and possible abnormalities in DA cell number in schizophrenics.

This research supported by Grants MH-33513, MH-30546, DA-2338, AA-0390, and the Biological Humanics Foundation.

30.4 EVIDENCE FOR THE SEGREGATION OF CATECHOLAMINES IN THE RAT CAROTID BODY. D. S. Christie* and J.T. Hansen. Department of Anatomy, The University of Texas Health Science Center, San Antonio, TX 78284.

Central to our understanding of arterial chemoreceptor transduction is the hypothesis that subtypes of glomus cells exist which selectively release either dopamine (DA) or norepinephrine (NE) as neuromodulators during chemoreceptor stimulation. However, evidence for the segregation of DA and NE to specific subtypes of glomus cells is indirect, based largely on total catecholamine (CA) content of the carotid body and on the morphology of the CA-containing dense-core vesicles. Therefore, to determine if there is a segregation of glomus cell CAs, we employed a pharmacologic paradigm used by Hess (Neurosci. 3:413, 1978) which depletes all carotid body CAs and then selectively regenerates DA while keeping NE levels significantly lowered. Reserpine (5mg/kg ip) significantly ($P < 0.01$) depleted CA levels and then, 24 hrs later, administration of L-dopa (100mg/kg ip) selectively regenerated DA to original control levels while NE remained significantly ($P < 0.01$) depleted. Analysis of carotid body CAs by HPLC 90 min after L-dopa administration validated this approach. However, ultrastructural stereology failed to demonstrate any significant difference in the concentration of glomus cell dense-core vesicles. Subsequently, we performed potassium dichromate cytochemical staining in an effort to distinguish those vesicles which still contained appreciable amounts of CA, presumably DA. Our preliminary results demonstrate that not all glomus cells are positively stained, suggesting that DA and NE may be segregated to subtypes of glomus cells. However, incubation of tissue for 24 hours in potassium dichromate at low pH (4.1) caused oxidation and poor morphological preservation. We currently are repeating our cytochemistry at higher pH (6.5), which should improve tissue morphology and enable us to more reliably identify which glomus cell subtype contains the presumptive DA staining. (Supported by USPHS Grants R01 HL25508 and RCDA K04-HL00680 to J.T.H.)

30.5 THE VENTRAL TEGMENTAL AREA OF THE MONKEY MESENCEPHALON. D.L. Felten and J.R. Sladek, Jr. (SPON: J.A. Clemens). Depts. of Anat., Indiana Univ. Sch. of Med., Indianapolis, IN 46223 and Univ. of Roch. Sch. of Med., Rochester, NY 14642.

The ventral tegmental area (VTA) of the rhesus monkey and squirrel monkey mesencephalon was examined with histofluorescence, Golgi-Cox, cresyl violet, and transmission and X-ray analytical E.M. to localize dopaminergic cell bodies, dendrites, and axons, to examine dendritic cytoarchitecture, and to localize synapses impinging upon dendrites and somas. Several zones of dopaminergic neurons were present in the VTA. In the ventral portion, medium-sized (20-35 um dia.) neurons were found within and dorsal to the interpeduncular nucleus. A few clusters of small neurons were noted near the midline. These ventral neurons were multipolar, with dendrites coursing in scattered directions. Fluorescent neurons were also abundant in the lateral region of the VTA, between the fascicles of the III nerve coursing through the ventromedial tegmentum medial to substantia nigra. The lateral VTA neurons were multipolar or bipolar, with many dendrites oriented vertically, in parallel with the III nerve fascicles. These dendrites were fluorescent; Golgi-Cox impregnation revealed small vertical clusters or bundles of dendrites. Axons of the ventral VTA neurons ran rostrally in a paramedian position in the ventral mesencephalic tegmentum, moved laterally to run just dorsal to the medial portion of substantia nigra, and then coursed through the lateral hypothalamus towards the basal forebrain. Both smooth and varicose axonal profiles were noted. A dorsal region of VTA neurons was also present in the midline. A narrow longitudinal wedge of cells extended upward from the base of the central gray between the paired columns of somatic oculomotor neurons. These small neurons (less than 20 um diameter), were present among a dense bed of catecholamine (CA) varicosities. Their dendrites were usually not fluorescent and did not form bundles. Their axons could only be traced for a short distance rostrally, and some appeared to head caudally. The neurons of the lateral VTA were examined with TEM for the topography of afferent synapses. The presence of CA in the VTA neurons was confirmed with the X-ray analytical technique of Wood (chromium tagging of glutaraldehyde-condensed CA). Numerous axo-dendritic synapses were identified (clear round and flat or pleomorphic, but very few dense core), and some dendro-dendritic appositions were present. Few axo-somatic synapses were found. The dendrites clustered into bundles, with dense afferent synaptic contacts, while the somas were relatively isolated from afferent synapses. These relationships may contribute to the regulation of these dopaminergic neurons which influence limbic and cortical functions. Supported by NIH grant RO1NS15677.

31.1 CO-OCCURRENCE OF SUBSTANCE P-LIKE AND CHOLECYSTOKININ-8-LIKE IMMUNOREACTIVITIES IN AN INTRINSIC FIBER SYSTEM OF THE CORTEX IN TURTLE. Anton Reiner and William D. Eldred. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

The pallium of the turtle telencephalon, from the septal area to the rhinal fissure, is arranged in a three-layered cortex: 1) a broad superficial molecular layer, 2) a thin cellular layer and 3) a thin subcellular layer. Three subdivisions of the cortex are generally recognized, a medial (cm), a dorsomedial (cdm) and a dorsal (cd). A lateral, noncortical extension of cd (termed the pallial thickening, PT) is often included as part of the cortical formation of turtle.

We have utilized LM and EM immunohistochemical techniques to characterize the distribution of substance P-like immunoreactivity (SPLI)(antibody obtained from Sera Labs, England) and cholecystokinin-8-like immunoreactivity (CCKLI)(antiserum obtained from M. Beinfeld) within the cortex and PT of turtle. With a simultaneous immunofluorescent labeling procedure (Erichsen et al., Nature, '82), we have found that both SPLI and CCKLI are present within an extensive fiber network of the cellular layer of the entire cortex and PT. The vast majority, if not all, of the fibers and terminals of this system contained both SPLI and CCKLI. Numerous SPLI-containing and CCKLI-containing neurons were observed in the molecular and cellular layers of cdm and cm. Cortical transection between cdm and cd resulted in the loss of both SPLI and CCKLI from cd and PT but not from cdm and cm. Thus, the intrinsic SP/CCK fiber system appears to originate largely from neurons of more medial portions of the cortex.

At the ultrastructural level, terminals containing SPLI show an identical labeling pattern to those containing CCKLI. In each, labeled terminals were filled with a diffuse cytoplasmic DAB reaction product and contained large labeled dense core vesicles (1300Å) and a few unlabeled large dense core vesicles (1300Å). Terminals containing SPLI and terminals containing CCKLI made conventional chemical synapses onto cell bodies in the cellular layer. Within labeled terminals, numerous small unlabeled vesicles (600Å) were clustered at synapses. The similarity in size of the SPLI-containing and the CCKLI-containing vesicles, as well as the small number of unlabeled large dense core vesicles, is consistent with the possibility that SPLI and CCKLI are present in the same vesicles.

The function of this intrinsic fiber system of turtle cortex is unclear. The present results, however, suggest that synaptic communication between this system and the cortical neurons may be complex and involve two neuroactive peptides and the additional unidentified substance(s) contained in the small unlabeled vesicles. This research was supported by NS-16857 to A.R. and EY-03801 to W.D.E.

31.2 RELATIONSHIP OF DOPAMINE AND CHOLECYSTOKININ IN MESOLIMBIC PROJECTIONS OF THE SUBSTANTIA NIGRA-VENTRAL TEGMENTAL COMPLEX. M. J. Iadarola, H.-Y.T. Yang and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Immunocytochemical studies have suggested the co-existence of dopamine (DA) and cholecystokinin (CCK) in neurons of the substantia nigra (SN)-ventral tegmental (VTA) complex, projecting to nucleus accumbens (NA) and olfactory tubercle (OT). We have studied this association with biochemical techniques in rats with unilateral 6-hydroxydopamine (6OHDA) infusion or mechanical hemitransection of the meso-limbic DA system. The OT, NA and caudate-putamen (CP) were assayed for CCK content (by radioimmunoassay) and tyrosine hydroxylase (TH) activity.

Ten to twenty days after hemitransection, the maximal loss of CCK was approximately 60% for both OT and NA. The CCK content of the CP remained unchanged even though TH activity was decreased 85-97% by the lesion.

The effect of 6OHDA lesions appeared to be highly dependent upon both the location and extent of the lesion. The maximal loss of CCK after 6OHDA lesions of the VTA was approximately 50% for both the OT and NA and was associated with nearly complete destruction of DA projections (>80% loss). The ratio of percent decrease in CCK to the percent decrease in TH was approximately 1:2 in both the OT and NA. Thus, almost 50% of the CCK content of OT and NA does not appear to be dependent upon the integrity of a DA projection.

Following 6OHDA lesions placed in SN, as much as a 50% loss of TH activity was obtained in the OT and NA; no change in CCK content was associated with these lesions. This suggests that although the more laterally placed DA cells in the vicinity of the SN send projections to the OT and NA, these projections contribute little or no CCK to these nuclei. As was observed with the hemitransections, no reduction of the CCK content of CP was obtained despite losses of TH of >85%.

In summary, our data suggest that 1) about 50% of the CCK content of NA and OT is not associated with mesolimbic DA neurons; 2) There is a small but significant contribution (to NA and OT) of cells lateral to VTA that contain DA but no CCK; 3) Nearly 70% of the DA innervation of the OT and NA may contain CCK; 4) The striatal content of CCK appears to be largely independent of the DA projections to this nucleus (in agreement with a report by Meyer et al., Science, 1982).

31.3 FACILITATION OF SPINAL MOTONEURON EXCITABILITY BY SEROTONIN AND SUBSTANCE P APPLIED AT THE SAME SITES WITHIN THE VENTRAL HORN. S. R. White. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3V6.

Serotonin (5HT) and substance P (SP) coexist in some neuronal cell bodies located in the medulla oblongata of the rat (Chan Palay, et al. Proc. natn. Acad. Sci. USA 75 1978, 1582; Hokfelt, et al. Neuroscience 3 1978, 517). Axons project from these somata to the spinal cord and form terminals in the ventral horn which also appear to contain both 5HT and SP (Gilbert, et al. Neuroscience 7 1982, 69). 5HT, applied iontophoretically, markedly enhances glutamate (GLU) excitation of spinal motoneurons (White & Neuman, Brain Research 188 1980, 119). Similarly, motoneuron responsiveness to dorsal root stimulation is enhanced by iontophoretically applied SP at some dose levels (Krivoy, et al. Brain Research 202 1980, 365). In the present study, the effects on motoneuron excitability of SP and 5HT applied at the same sites within the ventral horn were examined.

Multibarrel micropipettes were used for iontophoretic drug application and to record extracellular spikes from single alpha-motoneurons at L_4 or L_5 in rats. Automatic current balance was employed. Motoneuron spikes were evoked by cycling brief periods of GLU application. Stable responses to GLU were established and the effects of SP, 5HT and the acetic acid vehicle for SP were examined.

SP (25-50 nA, 60 sec) had qualitatively similar effects to 5HT (15-20 nA, 60 sec) on approximately 70% of the neurons tested. A brief period of inhibition of GLU-evoked activity was followed by a prolonged period of facilitation which outlasted current application by at least two minutes. Neither SP nor 5HT directly excited the motoneurons. For the remaining neurons, SP produced only facilitation of responsiveness to GLU with no inhibition. Preliminary results suggest that the facilitatory effects of SP and 5HT are additive. Acetic acid vehicle, alone, (30-50 nA, 60 sec) inhibited motoneuron firing during application, with recovery but no facilitation afterward. The effects of methysergide and putative SP antagonists are currently being examined.
(Supported by Medical Research Council of Canada.)

31.4 INCREASED EXCITATORY RESPONSIVENESS OF SPINAL NEURONS TO SUBSTANCE P AND 5-HYDROXYTRYPTAMINE IN p-CHLOROPHENYLALANINE-PRETREATED CATS. C. D. Raspantini*, S. Jeftinija*, T. L. Yaksh, V. L. W. Go*, A. A. Larson, and M. Randić (SPON: J. D. Blaustein). Dept. of Vet. Physiol. Pharmacol., Iowa State Univ., Ames, IA 50011; Dept. of Neurosurg. and Gastroent., Mayo Fnd., Rochester, MN 55905, and Dept. of Vet. Biol. Univ. of Minnesota, St. Paul, MN 55108.

The co-existence of 5-hydroxytryptamine (5-HT) and substance P (SP) in the same neurons of the brain stem raphé system and in its descending projections to the spinal cord has been demonstrated. Since the physiological significance of the presence of SP and 5-HT within the same spinal cord nerve endings is unclear, we have examined whether depletion of central 5-HT stores with p-chlorophenylalanine (pCPA) modifies responsiveness of spinal neurons to SP and 5-HT.

Thirty adult cats were pretreated with DL-p-chlorophenylalanine (350 mg/kg, i.p.) 72 hrs prior to the experiment. The activity of neurons in laminae I-VII of the lumbosacral and caudal spinal cord was recorded extracellularly. Microiontophoresis was used to study the effects of SP (3.7mM in 20mM acetic acid, pH 5.5, Beckman), 5-HT creatinine sulfate or HCl (50mM, pH 4-5), and norepinephrine bitartrate or HCl (0.2M, pH 3.8-4.7) on the spontaneous and evoked firing of the functionally identified neurons. The spinal cord concentrations of 5-HT and several neuroactive peptides (SP, neurotensin, cholecystokinin and vasoactive intestinal polypeptide) were also determined in control and p-CPA-pretreated cats.

Our results are based on data obtained from 189 neurons. Of these 92 were studied in the intact cats and 97 in the animals pretreated with p-CPA. SP(20-200nA for 2 min) caused excitation of 31% of the class 1 units (activated by sensitive mechanoreceptors) and about 70% of the class 2 (activated by nociceptors and sensitive mechanoreceptors) and class 3 units (activated by nociceptors only) in the intact cat spinal cord. However, in the cats pretreated with pCPA the number of units excited by SP was significantly increased, irrespective of the type of their sensory input. In addition, the proportion of nociceptive neurons depressed by 5-HT was decreased, as compared to controls. Units excited by 5-HT exhibited long-lasting and sustained responses. Three days following pCPA treatment, the 5-HT content of the dorsal and ventral halves of the spinal cord was reduced to 15.4% (0.11 ± 0.02µg/g fresh tissue) and 18.7% (0.13 ± 0.01µg/g) of control values, respectively. However, spinal levels of the peptides were not convincingly modified by the p-CPA treatment.

In conclusion, it appears that the 5-HT depletion increases excitatory responsiveness of all categories of cutaneous spinal neurons to SP and 5-HT.

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31.5 DESTRUCTION OF SUBSTANCE P AND SEROTONERGIC NEURONS BY EARLY POSTNATAL ADMINISTRATION OF 5,7 DHT. A. C. Towle,* R. A. Mueller, G. Breese, and J. Lauder. Biol. Sci. Res. Center and Depts. of Anesthesiology and Anatomy, UNC School of Med., Chapel Hill, NC 27514.

Substance P and serotonin are putative neurotransmitters which have been proposed to co-exist in some central neurons, particularly those in the ventral medulla which project into the spinal cord. In this study we have determined the effect of 5,7 DHT on the biochemical content and immunocytochemical localization of substance P and serotonin. Three-day-old rat pups were injected initially with DMI (20 mg/kg i.p.) then 1/2 hour later with 5,7 DHT (75µg intracisternal). Animals for immunocytochemistry were treated with colchicine 24 hours prior to perfusion with 4% paraformaldehyde 100 mM Borate pH 10.5 and processed for paraffin embedding. The antisera were localized using a bridge of biotinylated second antibody-avidin-biotinylated HRP. The serotonin antisera was raised in a rabbit immunized with a serotonin-hemocyanin conjugate and the monoclonal substance P antibody was purchased from Pel Freeze.

Early postnatal administration of 5,7 DHT results in a dramatic and irreversible loss of brain serotonin content when measured by reverse phase HPLC 2 weeks to 2 months later. Immunocytochemical evidence demonstrates the loss of most (80-90%) serotonergic cell bodies and terminals throughout the brain. This is in contrast with the many previous studies which have reported that following 5,7 DHT administration to adult rats the serotonergic neurons are "pruned" yet the cell bodies remain intact.

Interestingly, we have also observed that virtually all of the medullary neurons which contain both substance P and serotonin are destroyed by the 5,7 DHT treatment, yet neurons and terminals that appear to contain only substance P are not affected at all. In view of the large depletion of serotonin content and medullary substance P immunostaining it is somewhat surprising that substance P content is not similarly reduced in the spinal cord, medulla or midbrain when measured by radioimmunoassay. The 5,7 DHT treatment does reduce TRH content in the spinal cord by 50% indicating the destruction of TRH-serotonin neurons.

The observation that the substance P content remains constant, even with the complete and permanent destruction of the substance P-serotonergic neurons, suggests that either these neurons contribute relatively little to the total substance P content or that reinnervation occurs to take the place of the missing terminals.

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31.6 THE FORMATION OF CHOLINERGIC AND ELECTRICAL SYNAPSES BY RAT SYMPATHETIC NEURONS IN VITRO. D. Higgins*, L. Iacovitti+, T.H. Joh+ and H. Burton. Dept. Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110. +Lab. Neurobiol. Cornell Univ. Med. Coll., New York, NY 10021.

We have reported (Higgins & Burton, Neuroscience, in press) that sympathetic neurons from 21 day rat fetuses form electrotonic synapses when they are maintained in dissociated cell culture in a serum-free, chemically-defined medium (N_2, Bottenstein & Sato, PNAS 76:514-517, 1979). In these earlier experiments, the neurons received no exposure or a minimal exposure (< 2 hr) to a serum-containing medium (SM) (25% human placental serum, 10% chick embryo extract). We have now examined the effect of varying the length of exposure to SM on the properties of these neurons. Four groups of cultures of dissociated sympathetic neurons were established; group I was plated in N_2 supplemented with trypsin inhibitor (0.25%, 2 hrs) and then was maintained in N_2; group II was exposed to SM for 2 hrs and then was maintained in N_2; group III was exposed to SM for 48 hrs and then maintained in N_2; group IV was plated and maintained in SM. Fluorodeoxyuridine (10 µm) was used to kill non-neuronal cells. Choline acetyltransferase (CAT) activity ($\bar{X}$ ± SEM) was measured in monolayer cultures. Electrophysiological experiments and immunocytochemical staining for tyrosine hydroxylase were done during the 3rd and 4th weeks in vitro on neurons maintained on micro-islands of collagen (to enhance the detection of synapses).

In groups I & II only electrotonic synapses were observed; no CAT activity was detected (< .05 pmoles acetylcholine formed/neuron/hr) at either 3 or 6 weeks in vitro in group II. In group IV only chemical, cholinergic synapses were observed; the CAT activities were 2.2 ± 0.4 (3 weeks) and 9.0 ± 2.2 pmoles/neuron/hr (6 weeks). In group III both electrical and chemical, cholinergic synapses were observed; the CAT activities were 0.36 ± .02 (3 weeks) and 0.50 ± .07 pmoles/neuron/hr (6 weeks). In all 4 groups, more than 95% of the neurons were found to contain tyrosine hydroxylase molecules within their perikarya. Furthermore, in group III some of the neurons which contained tyrosine hydroxylase communicated with other neurons by means of both electrical and cholinergic synapses. We conclude: 1) that some neurons in vitro can simultaneously express parts of three different synaptic communication systems (cholinergic, catecholaminergic, electrical); 2) that even a brief exposure (48 hrs) to a cholinergic inducing medium is capable of causing a very long-lasting (6 wk) change in the properties of sympathetic neurons maintained in N_2. (Supported by NIH Research Grants NS 14416 and NS 09809 and a Fellowship from the Missouri Heart Association.)

32.1 COLOCALIZATION OF SUBSTANCE P, ACETYLCHOLINESTERASE, MUSCARINIC RECEPTORS AND ALPHA-BUNGAROTOXIN BINDING SITES IN THE RAT INTERPEDUNCULAR NUCLEUS. A. Rotter and D.M. Jacobowitz. Dept. of Pharmacology, Univ. of Calif., Irvine 92717 and Lab. of Clinical Science, NIMH, Bethesda MD 20205.

The interpeduncular nucleus (IPN) is a midline structure which receives a major, bilateral axonal input arising from the habenulae and passing through the fasciculi retroflexi. The evidence now available indicates that the neurons which make up this pathway contain acetylcholine (ACh) and substance P (sP). The presence of both neurotransmitters in the IPN raises the possibility of an interaction between the converging cholinergic and sP-containing systems. In order to clarify the spatial relationship between the two systems we have localized acetylcholinesterase (AChE) by acetylthiocholine histochemistry, sP by immunofluorescence methods and cholinergic receptors by autoradiography of receptor-bound tritiated quinuclidinyl benzilate or iodinated alpha-bungarotoxin (α-BuTX). The results have revealed considerable complexity in the spatial organization of the various neurochemically defined systems.

We have used the localization of AChE to define several zones of intense staining. In the rostral part of the IPN there is moderately high staining distributed uniformly throughout the nucleus. Further caudally, dense bands of staining are present as lateral zones (LZ) of the IPN which border on the medial lemniscus. The LZ is separated from a moderately stained median zone (MZ) by a lightly stained perivascular zone (PZ) which coincides with the previously described area containing crest synapses. Capping the MZ is a well demarcated region of densely stained neuropil extending to, but not continuous with the dorsal tips of the LZ. At the caudal end of the IPN intense AChE is found only in a narrow layer at the ventral margin of the nucleus. sP is present in high concentrations in the LZ and it's distribution is identical to that of AChE. The distribution pattern of muscarinic receptors and α-BuTX binding sites within the IPN correspond closely to that of AChE. High concentrations of both receptors are found in the LZ, MZ and the dorsal cap whereas the PZ is unlabeled. In addition, islands of high density labeling are found immediately lateral to the dorsal tip of the LZ. The receptors are localized postsynaptically since bilateral destruction of the habenulae does not diminish receptor density or change their pattern of distribution.

The results provide a morphological basis for an interaction between ACh and sP-containing axon terminal within the IPN, raise the possibility of nicotinic acetylcholine receptor function being modulated by sP and support the hypothesis that sP activity is terminated by AChE mediated hydrolysis.

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32.2 INTERRELATIONSHIP BETWEEN SEROTONINERGIC AND SUBSTANCE Pergic SYSTEM IN RAT BRAIN. P. Savard*, Y. Mérand*, P. Bédard, J.H. Dussault* and A. Dupont, Département d'Endocrinologie Moléculaire, CHUL and Département de Neurobiology, Hôpital de l'Enfant Jésus, Québec, Canada.

Thyroid hormone deficiency at birth is known to cause irreversible marked impairment of brain differentiation and lead to cretinism. Cell differentiation and synapse formation have been shown to be retarted in the hypothyroid state and accelerated by hyperthyroidism. Neonatal hypothyroidism led to a marked increase of substance P content mainly in nuclei known to be involved in brain substance Pergic system. Such enhancement was completely reversed by thyroxin treatment. Recently we also demonstrated that serotonin (5-HT) content was increased by postnatal thyroidectomy. On the other hand, coexistence of 2 putatives neurotransmitters, the biogenic amine 5-HT, and the polypeptide substance P (SP) has been demonstrated in some neurons of the CNS of the rat by immunofluorescence and various histochemical techniques. The aim of the present experiments was to investigate if the increased effect of neonatal thyroid deficiency on substance P was related to the high serotonin content in brain nuclei. Rats were made hypothyroid by injection of 125 μCi of carrier-free ^{131}I on the first day after birth. Half of this group of animals were subsequently treated with thyroxine (T_4). A third group of littermates were permitted to mature normally. For study the relationship of SP and 5-HT, we decreased the 5-HT metabolism with parachlorophenylalanine (PCPA) (i.p. 300 mg/kg) 48 and 24 hours before the sacrifice at day 45. Subsequently, SP content was measured by radioimmunoassay. The PCPA treatment increase the substance P content in nucleus dorsalis raphes and nucleus medialis raphes, the brain regions containing the cells bodies of 5-HT. Moreover, this treatment blocked completely the increased effect of neonatal thyroid deficiency on substance P content of brain nuclei, mainly substantia nigra, pars reticulata and nucleus interpeduncularis; both structures are involved in the better defined striatonigral and habenulo-interpeduncular tracts. The increase of substance P content of brain nuclei in postnatal thyroidectomy seems to be serotonin-dependent. This is the first demonstration of a pathophysiological interaction between substance P and serotonin at the brain level.

32.3 MODULATION OF SUBSTANCE P CONTENT OF RAT BRAIN NUCLEI FOLLOWING PHARMACOLOGICAL MANIPULATION OF SEROTONINERGIC SYSTEMS. Nicholas Barden*, Michel Daigle*, Vallier Picard* and Thérèse Di Paolo. (Spon. P. Leung). MRC Group in Moleculcar Endocrinology, Le Centre Hospitalier de l'Université Laval, Québec, G1V 4G2

Since substance P (SP) has been demonstrated to be co-localized with serotonin (5-HT) in the descending raphe system we have studied the effects of alterations of 5-HT metabolism in this and other brain areas. Brain nuclei were punched from frozen 300μm slices of rat brain and extracted with 0.1M $HCLO_4$ or 2M-acetic acid prior to assay, respectively, of 5-HT content by HPLC with electrochemical detection or SP content by specific radioimmunoassay. Ten days after injection of rats with the 5-HT neurotoxin, p-Cl-amphetamine (PCA, 10 mg/kg B.W., i.p.) or 3 days after 5-HT synthesis blockade with p-Cl-phenylalanine (PCPA, 300 mg/kg B.W., i.p.), the 5-HT content of all brain nuclei studied was reduced by a mean of respectively 50% and 81%. In PCA treated animals, no significant changes in SP content were noted, whilst following PCPA administration, the SP content of nucleus interpeduncularis was increased by 84% and that of substantia nigra pars reticulata was increased by 88% when compared to normal animals. Blockade of 5-HT receptors by methysergide (15 mg/kg for 5 days) did not significantly change 5-HT levels or turnover but resulted in 50-200% increases in the SP content of 10 of the 28 brain nuclei studied. Significant decreases in the SP content of numerous areas were seen following treatments (pargyline 30 mg/kg, 5-HTP 50 mg/kg or a combination of the two) which simultaneously increased 5-HT levels. These results ilustrate the modulation of distinct SP-containing systems of the rat brain by pertubation of central serotoninergic pathways and indicate a reciprocal relationship between the SP and 5-HT concentrations of numerous brain nuclei.

32.4 MOTOR EFFECTS OF INTRATHECAL INJECTIONS OF SUBSTANCE P AND 5-HT IN CHRONIC SPINAL RATS. L. Tremblay*, R. Maheux*, P. Bédard and M. Filion. Laboratoire de Neurobiologie, Hôpital de l'Enfant-Jésus, 1401, 18e Rue, Québec, Qué. G1J 1Z4.

Substance P (SP) was administered intrathecally (in the spinal subarachnoid space) to rats hypersensitive to intrathecal 5-HT and to intraperitoneal 5-HTP 15 days following complete spinalization or neurotoxic destruction of 5-HT pathways by 5-7-DHT. Large doses of SP (0.1 to 1.0 mg) depressed the spontaneous EMG activity of the paralyzed hindlimbs, blocked or prevented the large increase of EMG activity produced by 5-HT (or 5-HTP), and depressed or abolished nociceptive reflexes. These effects lasted at least 24 hours and up to several days after a single large dose. Smaller doses (below 0.1 mg) greatly increased the EMG activity. The increased activity was sometimes abruptly interrupted by a period of extinction lasting minutes. Thereafter it returned slowly to normal. During this time and for more than 24 hours after the administration of SP the responses to intrathecal 5-HT were greatly reduced; nociceptive reflexes, however, were not abolished. A depolarization block very likely explains the depressant action of large doses of SP. Indeed smaller doses induce a strong excitatory effect which sometimes leads to a block of short duration. However the depressant action of SP on the excitability of spinal neurons by 5-HT cannot always be explained by depolarization blocks since it is present when the EMG activity has returned to normal, 24 hours following the administration of SP. In conclusion SP has long term effects on the excitability of spinal neuron by 5-HT.

32.5 SYNAPTIC RELATIONS BETWEEN CORTICAL AND DOPAMINERGIC AFFERENTS AND ENKEPHALIN-CONTAINING NEURONS IN THE NEOSTRIATUM. J.J. Bouyer*, V.M. Pickel, T.H. Joh, R.J. Miller and D.J. Reis (SPON: B. Judd). Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021 and Dept. of Pharmacology and Physiological Sciences, Univ. of Chicago, Chicago, IL 60637

Pharmacological studies have shown that L-glutamate, the presumed neurotransmitter of cortical afferents to the neostriatum, may presynaptically release dopamine from nigrostriatal axons and also potentiate apomorphine-induced stereotypies (Scatton et al., Br. Res. 232:331, '82). By combining anterograde degeneration with electron microscopic immunocytochemistry, we sought to determine the neuroanatomical relation between cortical afferents, tyrosine hydroxylase (TH)-labeled dopaminergic terminals, and intrinsic neurons containing the morphine-like pentapeptide, Met^5-enkephalin in the neostriatum. Specific antisera to TH and to Met^5-enkephalin were localized by the peroxidase-antiperoxidase method in the neostriatum of adult rats 1-4 days following unilateral removal of the cerebral cortex. By light microcopy, varicose processes showing TH immunoreactivity had a similar intensity and distribution in the neostriatum homolateral and contralateral to the cortical ablation. In contrast, enkephalin-like immunoreactivity (ELI) appeared significantly more intense in perikarya and processes in the cortically deafferented versus intact neostriatum. By electron microscopy, about 35% of the total degenerating terminals showed an apposition of plasma membranes with terminals containing TH immunoreactivity. However, in most cases, specialized densities were not detected between the degenerating and TH-labeled axon terminals. Both the degenerating and TH-labeled terminals also formed asymmetric and symmetric contacts with unlabeled dendrites and dendritic spines. Electron microscopic analysis of degenerating boutons in relation to profiles showing ELI, indicated that enkephalin-containing dendrites received cortical afferents. The degenerating and enkephalin-labeled terminals were frequently in close proximity, but formed synapses predominately with unlabeled dendrites. These studies are consistent with a presynaptic interaction between cortical afferents and dopaminergic terminals, and further suggest that the enkephalinergic neurons may be stimulated by cortical afferents.

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32.6 FUNCIONAL INTERACTIONS BETWEEN CHOLECYSTOKININ AND DOPAMINE: A PHARMACOLOGICAL APPROACH. S.Govoni, E.Faccini*, G.Pasinetti*, C.Missale*, A.Rossi*, P.F. Spano*, M.Trabucchi. Dept. of Pharmacology and Pharmacognosy, University of Milan, Italy.

Recent immunocyto-chemical and electrophysiological studies indicate the existence of a functional relationship between cholecystokinin (CCK) and dopaminergic transmission. In order to gain more information on the interaction between CCK and dopaminergic neurons we measured the concentrations of Cholecystokinin immunoreactive material (CCK-IR) in structures of the nigro-striatal and of the mesolimbic dopaminergic system after pharmacological manipulation of dopamine synthesis and storage. In the same brain structures dihydroxyphenylacetic acid (DOPAC) concentrations were also measured after s.c. injection of Caerulein, a peptide having structure and biological activities similar to those of CCK, but more resistant to enzymatic cleavage.

Our results show that a treatment depleting Dopamine decreases the CCK-IR content in striatum and n.accumbens. The content of CCK-IR is decreased by 37% in rat striatum 24 hrs. after reserpine injection (6 mg/Kg s.c.). This decrease fails to occur in n.accumbens. The intravenous injection of α-methyl-para-tyrosin (α-MPT) decreases CCK-IR both in striatum and n.accumbens. On the other hand the subcutaneous injection of small doses of caerulein (20-50 ug/Kg) causes an almost complete reduction of the spontaneous motor activity and slightly reduces DOPAC content in striatum and n.accumbens. However at variance with the hypomotility induced by low doses of apomorphine, the decreased motor activity following caerulein injection (20 ug/Kg s.c.) is not antagonized by haloperidol pretreatment (50 ug/Kg s.c.).

Our results support the existence of a pharmacologically and, perhaps, physiologically relevant interaction between CCK and dopaminergic neurons. Nevertheless the difference between caerulein and apomorphine induced hypomotility indicate the need for further investigations of the neuronal mechanisms mediating caerulein actions after peripheral injection. It is tempting to speculate that the potent behavioural effects of CCK and CCK related peptides may be of some clinical relevance.

32.7 EFFECTS OF A LONG-TERM CHLORDIAZEPOXIDE ADMINISTRATION ON BIOGENIC AMINES CONTENT AND GAD ACTIVITY IN CAT BRAIN. A.G. Roberge, L. Vachon*, and N. Tremblay*. Dép. de Nutrition humaine (FSAA), et Dép. de Biochimie (Médecine), Université Laval, Ste-Foy, Québec, Canada, G1K 7P4.

In an attempt to precise the central neurochemical effects of chlordiazepoxide hydrochloride (CDP) on a neuroanatomical basis, cats underwent a 7 consecutive days treatment with CDP at doses of 0.4, 10.0, and 20.0 mg/kg/day, per os, and were killed 18 hr after the last administration. The serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NA), and dopamine (DA) endogenous levels were measured in 12 brain areas. Moreover, the enzymatic activity of glutamic acid decarboxylase (GAD) was assayed in some brain areas.

Few effects on the 5-HT, 5-HIAA, and NA contents were observed with a 0.4 mg/kg CDP treatment. These changes were found in the piriform lobe (amygdala), hippocampus, mesencephalon, and mesencephalon raphe nuclei. Moreover, the DA concentration was not affected in any structure assayed. On the other hand, the changes induced by the 10 and 20 mg/kg CDP doses were extended to many more structures. The effects observed after the two doses generally included an increased 5-HT content, a decreased 5-HIAA level, a high 5-HT:5-HIAA ratio, and a rised NA and DA concentrations. However, in some structures, after CDP administration, the NA level was found lowered, whereas the 5-HIAA content was enhanced.

GAD activity was reduced with a 0.4 mg/kg CDP treatment in the ventral mesencephalon, whereas the highest CDP dose 20 mg/kg induced an increased enzyme activity in this brain area. Moreover, the enzymatic activity was enhanced after both the 10 and 20 mg/kg CDP treatments in the hypothalamus.

These results show that a long-term CDP administration in cats induces localized changes in the endogenous content of biogenic amines at low doses, but much more extended effects were observed at high doses. They also reaveal that GAD activity is selectively affected by CDP treatment. Moreover, these findings are in agreement with a reducing action of benzodiazepines on the turnover and release of biogenic amines in the CNS, but also suggest that certain discrete areas are more involved by these changes, thus dissociating them from the rest of brain.

Finally, the variation observed in GAD activity also suggest an interaction between two types of neurotransmitters: biogenic amines and γ-aminobutyric acid (GABA) following a CDP treatment (Supported by a Grant (MA-6590) of the Medical Research Council of Canada. L. Vachon is holder of a studentship from the Fonds de la Recherche en Santé du Québec).

32.8 MECHANISM OF BENZODIAZEPINE ACTION ON RETINAL DOPAMINE NEURONS. C. W. Kamp and W. W. Morgan, Dept. of Anatomy, The Univ. of Texas Health Sci. Ctr. at San Antonio, Texas 78284.

Intraocular flurazepam (FLU, 0.5 or 1.0 μmole/eyeball), a water soluble benzodiazepine (BZ), caused a highly reproducible, dose-dependent inhibiton of the light-evoked enhancement of retinal dopamine (DA) synthesis (Kamp and Morgan, Eur. J. Pharmacol. 77: 343, 1982). The barbiturates, another group of CNS depressants, were shown previously to reduce retinal DA activity by potentiating a well-characterized, inhibitory GABAergic input to the dopaminergic cells. Since the BZ act in other areas of the CNS by enhancing GABAergic transmission, we proposed that the BZ reduced retinal DA activity by potentiating the GABAergic input to these cells. The results of studies to test this hypothesis are presented here.

Intraocular injections of the selective GABA antagonists, picrotoxinin or bicuculline methiodide were unable to reverse the actions of FLU even though the dosages proved potent enough to block the ability of the GABA agonist, muscimol (MU, 1.5 nmole/ eyeball), to suppress light-enhanced DA synthesis. A submaximal dosage of FLU was unable to enhance the inhibitory effects of a dosage of MU which could be readily potentiated by the barbiturates. Finally, the BZ antagonist, RO 15-1788 (4 μg/eyeball or 3.0 mg/kg, i.v.) could not block the FLU-mediated inhibition of DA sythesis.

Taken together these results imply that the effects of the BZ on retinal DA function may not be mediated by the classical GABA-linked BZ receptor found in other areas of the CNS or by a BZ receptor at all. (This project was supported by a PMA Foundation Grant to CWK and DA-00755 and NS14855 to WWM.)

32.9 DIFFERENT ADAPTIVE CHANGES OCCURRING IN CEREBRAL NORADRENERGIC AND SEROTONERGIC NEURONS AFTER PROLONGED STIMULATION OF GABA RECEPTORS. B. Scatton, B. Zivkovic*, L. Rouquier*, J. Dedek* and G.Bartholini*
Synthélabo-LERS, Research Dept. Paris, France.

Acute administration of the specific GABA receptor agonist progabide (100-1200 mg/kg ip) enhanced DOPEG-SO_4 and MOPEG-SO_4 levels and the rate of α-methyl-p-tyrosine-induced disappearance of norepinephrine (NE)-an effect antagonized by picrotoxin- in the hypothalamus, septal areas and olfactory tubercle but not in other NE-rich brain areas. Similar changes were observed in the hypothalamus after acute administration of other GABA agonists e.g. muscimol, depamide, THIP, kojicamine but not baclofen suggesting the involvement of a GABA-A type receptor. The GABA mediated enhancement of NE turnover does not appear to be connected to a direct effect of GABA on NE cell bodies as infusion of muscimol (0.1-1 µg) into the locus coeruleus failed to affect NE turnover ; it is most probably mediated indirectly via GABAergic synapses involved in the local circuits regulating noradrenergic neuron activity.

Enhancement of GABA synaptic activity decreases striatal serotonergic transmission. Thus, acute administration of progabide (200-600 mg/kg ip) decreases 1) the α-propyldopacetamide-induced disappearance of serotonin (5HT), 2) the amine accumulation caused by pargyline, 3) the accumulation of 5HTP following NSD-1015 in the rat striatum but not in other 5HT-rich brain areas.

After repeated treatment for 14 days with progabide (400 mg/kg, ip, bid), the initial increase in hypothalamic and septal NE utilization and DOPEG-SO_4 levels was no longer observed (tolerance). In contrast, repeated progabide treatment decreased striatal 5HTP accumulation more effectively than the acute treatment (reverse tolerance). Moreover, while the acute treatment failed to affect 5HT synthesis in cerebral cortex, repeated treatment with progabide decreased it. Tolerance with respect to increase in NE turnover is not connected to induction of drug-metabolizing enzymes or development of GABA receptor subsensitivity as 1) the progabide effects on 5HTergic transmission are preserved or even enhanced during repeated treatment, 2) no-cross tolerance between progabide and THIP or muscimol was observed. This tolerance is more likely related to adaptive changes of the mechanisms regulating NE release. In this respect however, the failure of repeated progabide treatment to affect the ability of clonidine to decrease hypothalamic or septal NE turnover excludes the occurence of a subsensitivity of α_2 adrenoceptors.

In conclusion, the present results indicate that cerebral NEergic and 5HTergic systems undergo opposite adaptive changes after prolonged stimulation of GABA synaptic activity. These differential adaptive changes may be connected to the different nature of the neuronal circuits involved in the regulation of NE and 5HT neuronal activity.

32.10 ACETYLCHOLINE INHIBITS DOPAMINE-SENSITIVE ADENYLATE CYCLASE OF RAT STRIATUM. M. Olianas*, P. Onali, N. H. Neff and E. Costa. (SPON: J.C. Byrd). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

We have recently shown that striatal muscarinic receptors are coupled in an inhibitory manner to adenylate cyclase (a.c.) and have suggested that occupancy of these receptors by endogenous effectors may modulate the sensitivity of this enzyme system to stimulatory inputs. In order to verify this possibility, we investigated the effect of acetylcholine (ACh) on the dopamine (DA)-sensitive a.c. assayed in a lysed synaptosomal preparation of rat striatum. In the presence of 20 µM GTP and 0.5 mM MgATP, DA stimulated the enzyme activity in a dose-dependent manner, with an apparent EC50 of 3-4 µM with a maximal effect (two fold increase) at a concentration of approximately 50 µM. When ACh was included in the reaction mixture, the stimulation of a.c. by DA was attenuated. Dose-response curves of a.c. activity to DA (1-100 µM), carried out in the absence and in the presence of 100 µM ACh, showed that ACh inhibited the net stimulation of a.c. by approximately 30-40% at all the concentrations of DA tested. Double reciprocal plots of the enzyme activity revealed that ACh decreased the Vmax of the DA-stimulated enzyme without significantly changing the Ka for DA. The inhibitory effect of ACh was completely antagonized by 2 µM atropine, which failed to affect the stimulation of a.c. by DA. Dose-response curve of a.c. to ACh (0.05-500 µM), carried out in the absence and in the presence of 3 µM DA, showed that the inhibitory effect of ACh on the DA stimulated activity was dose-dependent with an apparent EC50 of approximately 0.5 µM and reached a maximum at a concentration of 10 µM. ACh (100 µM) did not inhibit the stimulation of striatal a.c. by L-phenylisopropyladenosine (0.5 to 200 µM). These results indicate that occupancy of striatal muscarinic receptors by ACh inhibits the activation of a.c. by DA in a non-competitive manner. The opposite effects exerted by these neurotransmitters on the striatal a.c. system may be a part of the functional balance between the dopaminergic and the cholinergic inputs at the level of the basal ganglia.

32.11 ENHANCEMENT OF THE HALOPERIDOL-INDUCED SUPPRESSION OF FOOD-REINFORCED LEVER-PRESSING BEHAVIOR BY INTERACTION WITH α_2-ADRENORECEPTORS. J. A. Engel, K. Johannessen*, S. Liljequist* and M. Goldstein. Department of Pharmacology, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden, and Department of Psychiatry, New York University Medical Center, New York, N.Y. 10016.

Recent evidence indicates that the noradrenergic neuronal inputs regulate the activity of the dopaminergic neuronal systems. We have recently found that drugs affecting the α_2-adrenoreceptors alter the synthesis and turnover rate of the nigrostriatal dopamine-system. Thus the α_2-receptor antagonists SKF 64139 (7,8-dichloro-1,2,3,4-tetrahydroisoquinoline) and SKF 72223 (5,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline) increased the accumulation of DOPA and decreased the disappearance of dopamine following inhibition of its synthesis. The significance of these biochemical findings was further tested in behavioral experiments.

Inhibition of catecholamine synthesis with α-methylparatyrosine (α-MPT) was previously shown to potentiate the behavioral suppression caused by dopamine-receptor antagonists, in all probability due to inhibition of the compensatory increase in dopamine turnover induced by these drugs. In the present study we investigated the effects of SKF 72223 and the α_2-adrenoreceptor agonist clonidine on the haloperidol-induced suppression of food-reinforced lever-pressing behavior (fixed ratio 40:1) in rats. Small, behaviorally inactive, doses of both SKF 72223 and clonidine were found, in analogy with α-MPT, to enhance the haloperidol-induced behavioral effects. These results indicate that α_2-adrenoreceptor mechanisms may be involved in the regulation of dopaminergic activity. It is also suggested that the TIQ derivative SKF 72223 and clonidine may exert their effects on α_2-adrenoreceptors associated with different norepinephrine systems.

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32.12 ON-LINE DETECTION OF EXOCYTOSIS FROM ADRENAL CHROMAFFIN CELLS. B.G. Livett, P. Boksa* and †T.D. White, Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Québec, and †Dept. Pharmacology, Dalhousie University, Halifax, N.S. Canada.

The release of neurotransmitters and hormones from neuronal and endocrine cells is conventionally measured by sequential assays of the released transmitter or hormone by chemical, radiochemical or immunoassay techniques, following stimulation of the cells. The exocytotic release of catecholamines and opiates from adrenal chromaffin cells presents few problems when the cells are maintained as monolayer cultures firmly attached to a collagen substrate (Livett et al, Nature, 289:317-319, 1981) but is more difficult with cells in suspension culture which require centrifugation or filtration to separate the cells from the medium. These separation procedures are unsatisfactory in that they often result in high basal release values, take considerable time (2-3 min), and may damage and/or activate the cells. Chromaffin vesicles contain in addition to catecholamines (CA) large amounts of ATP which may be used as a sensitive marker for exocytosis. Adult bovine adrenal chromaffin cells prepared by collagenase digestion and Percoll fractionation (Livett et al, Nature, 278:256-257, 1979)were suspended in DMEM + 10% fetal calf serum and assayed in this medium for release of ATP, either immediately or after maintenance in suspension culture for 24h, by a modified firefly-tail luciferin-luciferase luminescence technique (White, J. Neurochem., 30: 329-336, 1978). Light output (maximum peak height) was proportional to ATP concentration over the range (10^{-9}-10^{-7}M). With this technique we were able to observe the initial time course of release of ATP during (rather than following) stimulation with various agonists, and its modification by a variety of antagonists and neuromodulators. Release of ATP induced by ACh, nicotine, K^+ and veratridine was Ca^{++}-dependent. The release of ATP paralleled that of CA and both showed desensitization at high agonist concentrations. SP produced an immediate and longlasting inhibition (IC_{50} 10^{-6}M) of nicotine- and ACh-evoked ATP secretion while having no effect on veratridine or K^+ evoked release. In contrast, Met-enkephalin and a number of opioid peptides were 100-1000 fold less potent than SP. Neither SP nor the opioids had any effect on basal release of ATP. The most potent opioid peptide was the endogenous "big" Met-enkephalin, the docosapeptide (BAM-22P) (Mizuno et al, BBRC, 97: 1283-1290, 1980). BAM-22P produced an enhanced and prolonged (20 min) basal release of ATP and also inhibited nicotine stimulated release of ATP by 42% at a concentration ($5x10^{-5}$M) where Met-enkephalin had no significant effect. This technique now provides a convenient inexpensive and rapid means of assessing the on-going interaction of drugs and endogenous neuropeptides with the process of secretion.
(Supported by Canadian MRC)

32.13 FAILURE OF ADENOSINE ANALOGUES TO PRODUCE BENZODIAZEPINE-LIKE SPECIFIC BEHAVIORAL EFFECTS. T. Gherezghiher*, D. Spencer, Jr., F. Elmesallamy, and H. Lal (SPON: I.M. Korr). Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

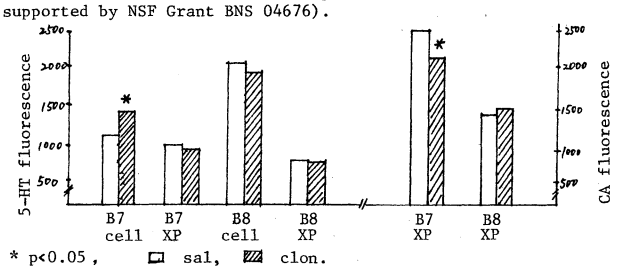
The purines, inosine and hypoxanthine, have been hypothesized to be endogenous ligands for the benzodiazepine receptor (Skolnick, et.al., Fed. Proc., 39: 3050, 1980). Adenosine has also recently been hypothesized to mediate the behavioral effects of benzodiazepines, since benzodiazepines inhibit adenosine uptake into cortical synaptosomes (Phillis, et.al., Gen. Pharmac., 12: 67, 1981) and adenosine-stimulated cAMP accumulation in-vitro (Traversa and Newman, Bioch. Pharm., 28: 2363, 1979). However, in-vivo evaluation of these ligands in terms of their similarity of behavioral effect to the benzodiazepines has not yet been carefully studied.

Blockade of the interoceptive discriminable stimuli produced by pentyletetrazol (PTZ) is a sensitive behavioral assay for the anxiolytic action of benzodiazepine activity (Lal, H. and Shearman, G., Ann. Rep. Med. Chem., 15: 15, 1980). We employed this assay to determine in-vivo interaction of adenosines with diazepam. Male hooded rats were trained in the drug discrimination procedure on an FR 10 food reinforcement schedule to press one lever when injected with PTZ (20 mg/kg) and another lever when injected with saline. After a reliable PTZ-saline discrimination was learned, prior injection of diazepam reliably blocked IDS produced by PTZ and the subjects chose the saline lever. When the adenosine analog 2-chloro-adenosine (2CA) and L-phenylisopropyl adenosine (L-PIA) were tested for benzodiazepine-like activity on this test, neither L-PIA (0.04 - 0.16 mg/kg) nor 2CA (0.16 - 0.32 mg/kg) blocked drug lever selection in the PTZ-injected rats in doses up to those producing profound sedation. These results indicate that activation of the adenosine A_1 receptor (Daly, et.al., Life Sci., 28: 2083, 1981) which leads to inhibition of adenylate cyclase and a fall in intracellular cAMP levels, does not produce benzodiazepine-like behavioral effects. It is therefore unlikely that inhibition of adenosine uptake and of adenosine-stimulated cAMP accumulation mediate the therapeutic efficacy of benzodiazepines in treating anxiety.

32.14 EFFECTS OF CLONIDINE ON MIDBRAIN RAPHE NEURONS IN RATS. E.H.Y. Lee* and M.A. Geyer, Dept. of Neurosciences and Psychiatry, Univ. of California, San Diego, School of Medicine, La Jolla, CA 92093.

By using our quantitative cytofluorimetry technique, we have previously reported that the dopaminergic agonist apomorphine selectively increased the intracellular level of serotonin (5-HT) in the dorsal raphe (B7) without affecting the median raphe (B8) (Lee, E.H.Y. & M.A. Geyer, Brain Res. Bull., In Press). In order to assess the influence of noradrenergic inputs to serotonergic neurons, we examined the effects of the alpha-adrenergic agonist clonidine on these raphe nuclei. Briefly, a measure of fluorescence fading using a computerized microspectrofluorimeter enables us to discriminate changes in 5-HT from changes in catecholamines and to discriminate intracellular from extracellular amines (Geyer et al., J. Pharm. Expl. Ther. 207:650-667, 1978). Forty-five minutes after intraperitoneal injections of saline or clonidine (50 µg/kg), male Sprague Dawley rats (225-250 g) were sacrificed, the midbrain raphe area was removed, freeze-dried, treated with formaldehyde, and embedded in paraffin. Microscopic measures were made in both the dorsal and median raphe nuclei in 8 micron sections. The results showed that as with apomorphine, clonidine elevated the intracellular 5-HT content in B7 significantly with no appreciable effect on serotonergic cells in B8. Conversely, clonidine significantly decreased extraperikaryal (XP) CA levels in B7, suggesting some alteration in CA release and/or turnover rate in this nucleus while CA levels in B8 were not affected.

The possible relevance of the ventral noradrenergic projections from locus coeruleus to B7 to the effects of clonidine is considered. Further studies including both clonidine and the noradrenergic antagonist piperoxane are in progress to determine the anatomical pathway and mechanism responsible for the effects of clonidine on dorsal raphe neurons. (This work was supported by NSF Grant BNS 04676).



32.15 NALOXONE-AMPHETAMINE INTERACTIONS: BIOCHEMICAL EFFECTS IN DOPAMINE TERMINAL REGIONS. Craig D. Applegate*, Ronald Kuczenski and Nancy J. Leith. (SPON: D. Schmidt). Dept. Pharmacology, Vanderbilt University School of Med., Nashville, TN 37232.

The ability of naloxone to antagonize the locomotor stimulant effects of low doses of amphetamine (AMPH) is well documented (Neuro. Sci. Abs., 6, 37, 1980). However, biochemical correlates for this pharmacological interaction have not been well defined. As the locomotor stimulant effects of AMPH have been shown to be dependent on the integrity of the mesolimbicocortical dopamine system (Brain Res., 201, 107, 1980), and as opiate agonists and antagonists have been shown to affect monoamine synthesis and release (J. Pharm. Pharmac., 30, 613, 1978) we have explored the biochemical consequences of naloxone-AMPH interactions in the mesolimbicocortical and nigrostriatal dopamine systems in an attempt to establish biochemical correlates for the behavioral interaction of these compounds.

Male Sprague-Dawley rats (N = 36) were administered saline or naloxone (1.0 mg/kg) immediately followed by saline or AMPH (1.0 mg/kg). Thirty minutes following drug administration, animals were sacrificed. The corpus striatum, substantia nigra, prefrontal cortex and nucleus accumbens were subjected to HPLC (electrochemical detection) analysis using a modification of the methods of Magnusson et al., J. Chromatog., 221, 1980. Using this technique we were able to determine the utilization of dopamine and serotonin in the brain areas examined.

Administration of 1.0 mg/kg of AMPH resulted in a characteristic increase in dopamine levels and decrease in the levels of its metabolites in the striatum and n. accumbens. Naloxone alone was not observed to affect any measure of dopamine biochemistry in these regions nor did naloxone alter the effects of AMPH on serotonergic function. However, combined injections of naloxone and AMPH resulted in a reversal of the AMPH induced decline in HVA levels in the n. accumbens (p = .019). This reversal of AMPH's effects by naloxone was not observed in any other brain region examined. The selective reversal in aspects of dopamine biochemistry in the n. accumbens following injections of naloxone and AMPH suggests this brain region may serve as the neural substrate for the behavioral effects of combined injections of these compounds.

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32.16 THE EFFECTS OF CHRONIC OPIATE TREATMENT ON α_2-ADRENERGIC RECEPTOR SYSTEMS. L. M. Vicentini, R. J. Miller* and M. J. Robertson*. Dept. of Pharmacological and Physiological Sciences, Univ. of Chicago, Chicago, IL 60637.

A growing body of literature suggests that opiate and α_2-adrenergic systems interact. For example, α_2 and opiate receptors appear to be colocalized in certain brain areas. Moreover clonidine, an α_2-agonist, has been shown to suppress some symptoms associated with opiate withdrawal in dependent subjects. Finally, a recent study (1) employing ^{3}H-clonidine (^{3}H-Clo) has indicated an increase in the number of α_2-receptors in the cerebral cortex of morphine addicted rats. We further investigated the effect of chronic opiate treatment on α_2-receptors by examining the binding of ^{3}H-yohimbine (^{3}H-YOH), an α_2-antagonist, to the cerebral cortex and whole kidney of morphine-addicted rats. In addition, we studied ^{3}H-YOH binding to NCB-20 neuroblastoma x glioma cells after chronic opiate treatment. This cell line possesses opiate receptors. Male Sprague-Dawley rats (250-300g) were implanted with 3 morphine pellets (75 mg base/pellet) and were sacrificed 72 hr later. The cerebral cortex and kidney were processed following the method of Perry and U'Prichard (2). NCB-20 cells were incubated with 1 µM etorphine or D-Ala^2, D-Leu^5-enkephalin or 10 µM morphine for 24-48 hr and binding of ^{3}H-YOH was determined by the method of Kahn et al. (3).

TABLE 1

^{3}H-Yohimbine

		Control		Addicted	
	n	K_D	B_{max}(fmol/mgpro)	K_D	B_{max}(fmol/mgpro)
Rat cortex	11	3.69 ± 0.72	66.6 ± 11.30	3.82 ± 0.56	67.22 ± 8.70
Rat kidney	5	2.35 ± 0.17	48.99± 7.37	2.44 ± 0.14	53.82 ±10.50
NCB-20	5	4.50 ± 0.49	73.66± 10.26	4.68 ± 0.69	62.03 ±11.03

Binding determinations using ^{3}H-Clo yielded essentially similar results as those obtained with ^{3}H-YOH. These data indicate that 1) α_2-adrenergic receptors exist on NCB-20 cells and 2) no significant alterations in either α_2-receptor affinity (K_D) or number (B_{max}) after chronic opiate exposure were apparent in any of the systems examined.

(1) Hamburg, M. and Tallman, J.F. (1981). Nature, 291, 493.
(2) Perry, B.D. and U'Prichard, D. (1981). Eur. J. Pharmacol., 76, 461.
(3) Kahn, D.J. et al. (1982). Mol. Pharmacol., 21, 17.

32.17 TRANSMITTER INTERACTIONS IN THE CEREBELLUM OF THE RAT. R.S. Flint* and W.J. McBride. Depts. of Psychiatry & Biochemistry, Inst.Psych. Res., Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

Examination of the depolarization-induced release of endogenous amino acids has indicated transmitter roles for GABA, glutamate (Glu) and aspartate (Asp) in the cerebellum of the rat (Flint et al., J. Neurochem. 37: 1425, 1978). Other investigations have implied that acetylcholine (Kan et al., Brain Res. 146: 221, 1978) and norepinephrine (NE) (Bloom et al., Brain Res. 25: 501, 1971) may participate in the process of neurotransmission within this region as well. In order to study the interactions among these putative transmitter systems, we examined the changes in the K^+-stimulated, Ca^{2+}-dependent release of endogenous NE, GABA, Glu, and Asp in response to exogenously added NE, GABA, Glu, Asp, and the cholinergic agonist, carbachol. The cerebella from two adult Wistar rats were removed and sliced longitudinally into 300 x 300 µm sections. These slices were combined and then suspended in Sephadex in polypropylene columns for superfusion. Initially, the K^+ concentration was varied to determine what level would yield a submaximal stimulated release, thus, providing a sufficient range for the detection of either inhibitory or excitatory effects. A K^+ concentration of 30-35 mM was found to be adequate for this purpose. The indicated transmitter candidates were administered individually at a concentration of 100 µM in the superfusion media containing the elevated level of K^+ in both the absence and presence of Ca^{2+}. The stimulated release of NE was significantly potentiated by GABA (130% of control; $p<0.05$); carbachol tended to slightly increase the release of NE (113%) as did Asp (119%; N= 3), while Glu had no effect. However, addition of NE caused a small decrease in the release of Asp (84% of control; N=3) while GABA potentiated its release (290%; $p<0.05$); carbachol had no effect on the release of Asp. GABA also increased the K^+-stimulated release of Glu (150% of control; $p<0.05$). NE and carbachol did not alter Glu release, nor did they effect the stimulated release of GABA. All observed changes were dependent on the presence of Ca^{2+}. The effects on the release of NE by carbachol, GABA, and Asp are presumably mediated through a direct interaction with presynaptic receptors on the terminals of the noradrenergic projection into the cerebellum. However, these data do not rule out interneuronal involvement. The stimulatory effect of GABA, although discordant with electrophysiological studies, is consistent with a previous study in this area of the CNS (Gallo et al., Brain Res. 205: 431, 1981). These results suggest that the transmitter interactions and neural circuitry within the cerebellum may be more complex than previous studies have so far indicated. (Supported by MH 00203 and The Indiana Public Health Trust).

32.18 ACETYLCHOLINE-MEDIATED INHIBITION OF BASAL AND VASOACTIVE INTESTINAL PEPTIDE-STIMULATED ADENYLATE CYCLASE IN RAT GH3 PITUITARY CELLS. J.P. Schwartz, M. Olianas* and P.L. Onali. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

The rat GH3 pituitary cell line has been used as a model system to study the biochemical events required for regulation of prolactin secretion. Many of the agents known to regulate prolactin secretion from anterior pituitary in vivo, including thyroliberin (TRH) and vasoactive intestinal peptide (VIP), also affect prolactin release in this cell line. VIP and TRH have been shown to stimulate adenylate cyclase in both GH3 and anterior pituitary cells: however, the role of the resultant increase of cyclic AMP in the secretion of prolactin has not yet been clearly elucidated. Acetylcholine (ACh) has been reported to inhibit prolactin secretion from GH3 cells and thus offered the possibility to examine further the relationship between adenylate cyclase activation and prolactin secretion. We now report that ACh can inhibit both basal and VIP-stimulated cyclase in GH3 cells, thus supporting the concept that cellular cyclic AMP levels correlate with prolactin secretion rates. VIP stimulated the adenylate cyclase of GH3 cells 11-14 fold, with a K_a of $\sim 2 \times 10^{-8}$M. The inclusion of 100 µM ACh depressed the VIP stimulation of cyclase by 60% in a non-competitive manner: the K_a for VIP in the presence of ACh was 2.5×10^{-8}M. ACh also inhibited basal adenylate cyclase activity, with a maximal inhibition of 35-40%. The K_i for ACh inhibition of basal adenylate cyclase was 3×10^{-7}M and a comparable K_i was determined for the ACh inhibition of VIP-stimulated cyclase. These effects of ACh were blocked by 1 µM atropine, suggesting that ACh is acting through a muscarinic receptor. These results provide support for the concept that activation and/or inhibition of the adenylate cyclase of mammotrophs by neurotransmitters or neuromodulators is directly related to the effects of these agents on prolactin secretion.

32.19 SEROTONERGIC TERMINALS IN THE NUCLEUS TRACTUS SOLITARIUS: ULTRASTRUCTURE AND SYNAPTIC ASSOCIATIONS WITH CATECHOLAMINERGIC NEURONS. V.M. Pickel, J. Chan*, T.H. Joh and A. Beaudet. Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021 and Montreal Neurological Inst., McGill Univ., Montreal, Canada.

Fluorescence histochemistry and immunocytochemistry have shown that terminals containing serotonin, 5-hydroxytryptamine (5-HT) are localized within the nucleus tractus solitarius (NTS), including the catecholaminergic neurons of the A2 region. In this study, immunocytochemistry and radioautography are combined in order to determine: (1) the ultrastructural morphology of 5-HT terminals in the NTS and (2) whether the 5-HT terminals form synapses with catecholaminergic neurons in the A2 region. Adult rats were pretreated with a monoamine oxidase inhibitor and subjected to a 2 h intraventricular infusion of 50 nM ^{3}H-5HT. The brains were fixed by intraaortic arch infusion of a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde. Twenty micron vibratome sections were collected throughout the rostrocaudal NTS, then immunocytochemically labeled with specific antiserum to serotonin (Immunonuclear Corp.) or tyrosine hydroxylase (TH), an enzymatic marker for catecholamines. Alternate immunocytochemically-labeled sections were processed for light or electronmicroscopic radioautography. By light microscopy, punctate clusters of reduced silver grains indicating uptake of ^{3}H-5-HT were distributed throughout the medial and commissural portions of the NTS. The distribution of radioautographically labeled varicosities generally paralleled the distribution of peroxidase labeling for serotonin and superimposed certain dendrites immunocytochemically labeled for TH. By electron microscopy, the serotonergic terminals identified either by radioautography or immunocytochemistry exhibited a dark cytoplasmic matrix and contained numerous, small, clear and a few large, dense vesicles. These terminals generally formed either asymmetric or symmetric axodendritic synapses. However, a few of the serotonergic terminals formed symmetric junctions with perikarya or failed to show membrane specializations in four or more serial sections. In favorable longitudinal planes of section, many of the apparently "non-synaptic" terminals were " boutons en passant" which formed symmetric axodendritic contacts at some point along their length. The perikarya and proximal dendrites receiving the serotonergic terminals usually did not have TH immunoreactivity. However, more distal dendrites and dendritic spines of the catecholaminergic neurons labeled with TH directly received terminals which incorporated ^{3}H-5-HT. These studies demonstrate an extensive network of serotonergic terminals which predominately contact the soma and proximal dendrites of non-catecholaminergic neurons and the more distal dendrites of catecholaminergic (A2) neurons within the NTS.

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32.20 GABA TURNOVER IN RAT STRIATUM: EFFECTS OF GLUTAMATE AND KAINIC ACID. O. Giorgi*, J. L. Meek, and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington D.C. 20032.

Previous work from this laboratory has demonstrated that the increases in GABA content after local injection of the irreversible GABA-T inhibitor gabaculine provides an estimate of GABA turnover that can be useful for comparative purposes (Forchetti and Meek, J. Neurochem. 38:1336-1341, 1982). As part of a study on the interactions of neurotransmitters and GABA in the striatum, we have further characterized this technique, and examined the effects of two excitatory aminoacids (kainic acid and glutamate) on GABA accumulation elicited by local injections of gabaculine. GABA content in the striatum was measured by HPLC. GABA-T activity was assayed in striatal homogenates using saturating concentrations of GABA, alpha-ketoglutarate and pyridoxal phosphate. The rate of formation of product (glutamate) was measured by HPLC. Drugs were injected stereotaxically into the striatum of ether anesthetized rats in a total volume of 2 µl over 3.5 min.

A dose of gabaculine (20 µg) which induced a maximal accumulation of GABA caused only a 50% inhibition of GABA-T 60 min after injection. With a 100 µg dose of gabaculine, GABA-T inhibition was 93% at 60 min. We used this dose subsequently to prevent possible artifacts due to incomplete inhibition. Under these conditions, GABA accumulation (25 nmol/mg prot /hr) was linear with time for at least 60 min.

One week after injection of kainic acid (1 µg) to destroy intrinsic striatal neurons, the GABA content was descreased by 55%. The accumulation of GABA after gabaculine was decreased by 60%. The results indicate that the majority of the GABA which accumulates arises from neurons sensitive to kainic acid.

A major neuronal afferent to the striatum is from the cortex. This excitatory pathway may be glutamatergic. We examined the acute effect on GABA accumulation of glutamate and of kainic acid, an excitatory, non-metabolizable, non-accumulated amino acid. When gabaculine was injected simultaneously with kainic acid (4.7 nmol) the accumulation of GABA was linear with time, and significantly greater than controls (42 vs 30 nmol/mg prot/hr). Glutamate itself was able to significantly increase GABA accumulation after gabaculine. A dose of 1 µmol glutamate caused an accumulation that was 59% greater than in animals receiving only gabaculine. The increase in the turnover rate of GABA most probably reflects the increase in neuronal activity induced by these excitatory aminoacids.

33.1 IDENTIFICATION AND PHARMACOLOGICAL SENSITIVITY OF A TRANSIENT OUTWARD CURRENT IN THE SOMATA OF CULTURED MAMMALIAN CENTRAL NEURONS. Jeffery L. Barker and Michael A. Rogawski (SPON: T.G. Smith). Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20205.

Molluscan neuron somata possess an outward current species, I_A, which becomes activated upon depolarization from potentials more negative than resting potential and which displays rapid time-dependent inactivation (J.A. Conner and C.F. Stevens, J. Physiol. 213: 21, 1971; E. Neher, J. gen. Physiol. 50: 36, 1971). It has been suggested that I_A serves to regulate the frequency of repetitive spike firing during sustained depolarization by retarding the decay to threshold of the hyperpolarizing afterpotential following a spike. In the present study, we identified a similar outward current in mammalian cultured neurons that seems to have a comparable functional role. Membrane currents were recorded under voltage clamp from cultured mouse spinal cord neurons using separate voltage-sensing and current-passing microelectrodes. The cells were bathed at 21° C in Hank's balanced salt solution containing tetrodotoxin (TTX; 1-3 μM) to block the voltage-sensitive Na^+ current. Cationic salts and drugs were applied extracellularly by pressure ejection from blunt micropipettes. Voltage steps to -50 mV or above from holding potentials of -60 mV or less elicited a transient outward current which peaked within 5-15 msec and decayed with a time constant of ~25 msec. Hyperpolarizing conditioning steps to -70 mV of as short as 10 msec could remove inactivation produced by holding at -50 mV. Inversion of the current relaxation following a brief depolarizing command occurred at -60 to -70 mV, suggesting that K^+ is the charge carrier of the current. As is the case in molluscan somata (S.H. Thompson, J. Physiol. 265: 465, 1977), high concentrations of 4-aminopyridine (4-AP; 2-10 mM) blocked the transient current in a dose-dependent fashion whereas tetraethylammonium ion (TEA; 25 mM) or Co^{2+} (20 mM) were without effect. In contrast to I_A, the delayed K^+ conductance (I_K) was unaffected by 4-AP, although it was highly sensitive to TEA. Consequently, spike broadening was observed with TEA but not 4-AP. To examine the functional role of I_A, 4-AP (10 mM) was applied to spinal neurons in TTX-free medium during the passage of a long depolarizing current step at rheobase. The drug induced the cells to generate repetitive action potentials by facilitating the rise of the electrotonic potential between spikes. These results indicate that mammalian central neurons possess a transient outward current qualitatively similar to that of molluscan neurons. Interference with I_A should lead to abnormal neuronal discharge, and this might, in part, contribute to the convulsant activity of 4-AP. Moreover, physiological modulation of I_A could be an important factor in the regulation of neuronal firing in the mammalian central nervous system.

33.2 4-AMINOPYRIDINE ENHANCES VOLTAGE-SENSITIVE CALCIUM ENTRY IN CULTURED MAMMALIAN CENTRAL NEURONS. Michael A. Rogawski and Jeffery L. Barker. Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20205.

4-Aminopyridine (4-AP) produces a powerful enhancement of quantal neurotransmitter release at the neuromuscular junction and at central and peripheral synapses. This effect has been attributed to presynaptic spike broadening resulting from a blockade of voltage-sensitive K^+ channels. However, the effects of 4-AP on transmitter release occur at concentrations below those which interfere with K^+ conductances. Therefore, the alternative hypothesis that 4-AP promotes the influx of Ca^{2+} through voltage-sensitive channels has been proposed, although no direct supporting evidence has as yet been obtained. To distinguish between these two alternatives, we carried out intracellular recordings from the somata of mouse spinal neurons in dissociated culture. Current recordings were obtained under voltage clamp with separate voltage-sensing and current-passing microelectrodes. Low concentrations of 4-AP (0.04-1 mM) applied by pressure ejection from micropipettes markedly enhanced the frequency and amplitude of synaptic potentials recorded in these cells. At these concentrations, 4-AP had no effects on spike duration or on the delayed outward current (I_K). When the cells were bathed in medium containing tetrodotoxin (3 μM) to block I_{Na} and tetraethylammonium ion (25 mM) to block I_K, a highly voltage-dependent, slowly inactivating inward current was revealed. This current became activated upon voltage clamp steps to -30 mV from holding potentials more negative than -50 mV. The following evidence indicates that the charge carrier of this inward current is Ca^{2+}: (1) the current is absent in Ca^{2+}-free medium but is expressed upon pressure ejection of Ca^{2+} (10 mM), (2) the current is blocked by Co^{2+} (10-20 mM), Mn^{2+} (15 mM) and Ni^{2+} (15 mM), (3) the current is present when the cells are bathed in medium containing Ba^{2+} (2 mM) instead of Ca^{2+}, and (4) the tail of the current diminishes in amplitude upon repolarization to more depolarized potentials but does not invert. In 2 mM Ca^{2+}-containing medium, pressure ejection of 4-AP (0.1-10 mM) markedly enhanced the amplitude and slowed the decay of the Ca^{2+} current, whereas in Ca^{2+}-free medium or in the presence of Co^{2+} there was no detectable effect on the voltage-current characteristics. These results demonstrate that 4-AP can facilitate a voltage-dependent Ca^{2+} current at concentrations more than 10-fold lower than required to block K^+ currents (see companion abstract). If similar effects occur at the nerve terminal, this could account for the facilitatory effect of 4-AP on quantal release.

33.3 CHARACTERISTICS OF A PERSISTENT MUSCARINE-SENSITIVE POTASSIUM CURRENT IN CULTURED RAT SYMPATHETIC NEURONS. J. E. Freschi. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

I previously described the membrane currents seen under two-electrode voltage clamp of neurons in primary cultures of rat superior cervical ganglia (SCG) (Freschi, J. E., Soc. Neurosci. Abstr. 7:901, 1981). I describe here further properties of a persistent muscarine-sensitive potassium current (M-current, Brown and Adams, Nature 283:673, 1980).

Depolarizing steps from the resting potential activated an outward current that exponentially deviated from the leakage current at potentials negative to those expected for classical outward rectification. The outward current activated in this range was better studied with hyperpolarizing step commands from holding potentials between -30 and -60 mV. Such commands caused a small inward relaxation with a time constant (τ) of 50-100 msec (37°). Repolarization then evoked an outward current with a τ always larger than that of the tail current. The inward relaxations became faster and smaller with increasing hyperpolarizing commands and became outward at potentials more negative than -80 mV. This reversal potential for M-current tails was dependent on external potassium concentration. The ohmic current jump at the end of voltage steps was smaller than that at the beginning, suggesting that hyperpolarization moves the cell to a lower conductance state. In fact, M-conductance reached a minimum at about -70 mV from a maximum value of 50 nS at about -20 mV. This conductance activation curve could be fitted by an exponential equation using constants that suggest that a single tetravalent gating particle controls the M-channel conductance state. The M-current was abolished by muscarine (1 μM) and reduced by TEA (30 mM). Cobalt had no consistent effect on M-currents.

These data agree with M-current analyses previously done with bullfrog sympathetic neurons and with isolated adult rat SCG neurons studied with single-electrode voltage clamp (Constanti, A. and Brown, D. A., Neurosci. Lett. 24:289, 1981). Minor differences between these studies will be discussed.

33.4 I_h: AN INWARD CURRENT UNDERLYING ANOMALOUS RECTIFICATION IN MOUSE SENSORY NEURONS. G.Westbrook*and M.Mayer.* Lab.Devel.Neurobiol., NIH, Bethesda,Md.20205(SPON: M.H. Whitnall)

Anomalous rectification occurs in many neurons and is observed with voltage recording as a membrane resistance decrease on hyperpolarization, or as a membrane resistance increase on depolarization. Recent evidence suggests that in some neurons membrane depolarization activates a persistant inward current producing an apparent membrane resistance increase under current clamp, but this was not seen in DRGs. However, anomalous rectification was prominent during membrane hyperpolarization.

We have investigated the mechanism of anomalous rectification evoked by membrane hyperpolarization in tissue cultured mouse DRG neurons with a two electrode voltage clamp. Hyperpolarizing commands from the resting potential(-50 to -60mV) evoked slow inward current relaxations. The activation variable of this current(I_h) is close to zero at -60mV and one at -100mV. The time constant of the I_h relaxations and their envelopes was strongly voltage dependent. Ih appears to be a mixed Na^+-K^+ current since inward current relaxations evoked from a holding potential of -50 or -60 mV in 4.5,14.5, or 25mM K^+ were strongly suppressed in Na^+-free solution, and the inward tail currents were abolished; or in 4.5mM K^+ reversed.

Cs^+(0.2-10mM) reversibly blocked I_h and prevented the time dependent sag seen in hyperpolarizing electrotonic potentials(i.e. anomalous rectification). I_h was not blocked by TTX, Mn^{++}, or Ba^{++} and is thus a distinct current from that generating "anomalous rectification" during membrane depolarization. It is, however, strikingly similar to the cardiac current, I_f or I_{K2}. Adams and Halliwell(J.Physiol.,324:62P,1982) have also described inward relaxations in hippocampal neurons, activated over a similar voltage range, and distinct from the M current. The original descriptions of anomalous rectification in neurons suggested that a membrane resistance drop occurred on hyperpolarization. In DRGs under voltage clamp, measurement of the membrane chord conductance indicates that a genuine conductance increase does occur on hyperpolarization, due to the activation of I_h, confirming earlier speculation.

At present it is unclear how the time and voltage dependent current I_h relates to the mechanism generating anomalous rectification in muscle, where the channels carrying inward K^+ current show strong rectification in the inward direction. (MLM is a Harkness Fellow.)

33.5 PROPERTIES OF DELAYED RECTIFIER K CHANNELS IN NEUROBLASTOMA CELLS. Fred N. Quandt and Toshio Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., 303 E. Chicago Ave., Chicago, IL 60611.

Presently, there are few reported studies concerning the properties of single delayed-rectifier K channels. We have measured currents through individual K channels of this type from membranes of N1E-115 neuroblastoma cells.

Recordings of currents using the gigaohm-seal, patch clamp technique were made from intact or excised membranes. Outward currents due to the opening of voltage-dependent channels occur in response to depolarizations to potentials less negative than -20 mV. This potential is at the foot of the conductance-voltage curve for the delayed-rectifier K currents recorded from the whole cell ($g_K/\bar{g}_K$=0.02). In outside-out patches, these currents were blocked by the addition of 15 mM tetraethylammonium to the external perfusate, a procedure which blocks the delayed-rectifier K channels in these cells. These channels did not appear to require the presence of internal Ca, since they were recorded from outside-out patches using a K glutamate internal solution to which 20 mM EGTA had been added; or from inside-out patches using a 150 mM KF, 0 mM Ca internal solution.

The conductance of the open state of these channels was 16 pS in one typical case (10°C). The conducting state of the channel usually exhibited multiple, short-duration transitions toward the closed conductance level, similar to those reported for K channels in squid axons (Conti and Neher, Nature 285, 140, 1980). A typical mean open time (-10 mV, 6°C) was 43 msec. K currents recorded from the whole cell inactivate over a period of seconds. Prolonged depolarizations applied to membrane patches indicated that the probability of a K channel opening declined over a similar period of time. Inactivation can be explained by the reduced probability.

A second type of outward current due to channel opening, which was smaller in amplitude, was frequently observed during depolarizations. Both types of conducting states were observed in the same recording, and occur in either cell-attached membranes or excised, inside-out membranes. Both populations of conducting states were reversibly blocked by replacing internal K with Cs in inside-out membrane patches, indicating that K is the normal current carrier for each. The conductance ratio for the two populations is estimated to be 0.3, assuming the same reversal potential for each. This second population is often observed, although not exclusively, after inactivation of the larger amplitude conducting state. The two populations could represent separate open states for a single type of channel, or separate types of delayed rectifier K channels. Supported by NIH grant NS14144.

33.6 DECAY OF CALCIUM CURRENTS IN RAT SKELETAL MUSCLES P. Lynn Donaldson and Kurt G. Beam* Department of Physiology and Biophysics University of Iowa Iowa City, IA 52242

Currents were measured in rat omohyoid muscles using the three microelectrode voltage clamp technique (Adrian, et al., J. Physiol. 208:645, 1970). When the larger and faster ionic currents carried by sodium and potassium in rat omohyoid muscles were blocked, a slow inward Ca current remained. (External solutions contained (mM): TEABr (146), CsBr (5), $CaAc_2$ (2-10), $MgAc_2$ (1), HEPES (10), sucrose (400), TTX (1 uM), and 3,4-diaminopyridine (5).) Ca currents were strongly affected by temperature, being barely detectable at temperatures less than 15° C and often regenerative at temperatures above 30° C. Most currents were recorded at temperatures of 24-27° C. Inward Ca currents decayed during a maintained depolarization and were sometimes succeeded by a late outward current of variable magnitude. In some muscle fibers, the inward Ca current was blocked by cadmium (0.2-0.5 mM) and the outward current was reduced. In other fibers, the late outward current of control records was nearly the same as the outward current which remained after the inward current was blocked. It, therefore, appeared that the decay of inward currents is a consequence of Ca channel inactivation, activation of a Ca-independent outward current, and, in some fibers, a Ca-dependent K current. The addition of nickel or manganese (5 mM) altered the inward current in a time-dependent manner. Bathing muscles in solutions containing these cations and Ca, initially reduced the rate of inward current decay and the apparent steady-state level of the late outward current with little effect on the peak current. Currents recorded after a longer exposure to Ni or Mn had reduced inward peak amplitudes. The time-dependence of these effects suggests two spatially separate sites of action. The early effect of Mn and Ni may be to decrease either Ca-dependent or Ca-independent late outward current. At later times, these cations block inward Ca current. Barium, substituted for Ca, slowed the decay and reduced the amplitude of the inward current. When Ca was returned to the bath, the inward currents were larger than the currents prior to Ba.

This work was supported by MDA and NS14901.

33.7 USE OF AEQUORIN TO DETERMINE CHANGES IN INTRACELLULAR FREE Ca^{++} IN MURINE NEUROBLASTOMA CLONE N1E-115 CELLS. R. Michael Snider, Elliott Richelson and John Blinks. Departments of Pharmacology and Psychiatry, Mayo Foundation, Rochester, MN 55905.

A rise in intracellular free calcium ion concentration [Ca^{++}] is implicated in a variety of neurobiological processes, including neurotransmitter release and receptor-mediated cyclic GMP formation. One of the most satisfactory tools for monitoring changes in [Ca^{++}] is the bioluminescent protein aequorin, which changes the intensity of its light emission in response to variations in [Ca^{++}] within the physiological range. Aequorin can be microinjected into small cells only with great difficulty, however, and to date the only neuronal cells which have been studied with aequorin are the giant nerve cells of invertebrates (see Blinks, Techniques in Cellular Physiology-Part II, P126:1-38, 1982).

Murine neuroblastoma clone N1E-115 cells are well suited for studying intracellular [Ca^{++}] and relating the results obtained to neuronal physiology. These cells are thought to have both voltage-sensitive (Study et al., Proc. Natl. Acad. Sci. 75:6295-6299, 1978) and voltage-insensitive (Richelson and El-Fakahany, Biochem. Pharmacol. 30:2887-2891, 1981) Ca^{++} channels. Moreover, activation of guanylate cyclase is hypothesized to result directly from a rise in intracellular [Ca^{++}].

Aequorin was loaded into cells by making them reversibly hyperpermeable (Winegrad, J. Gen. Physiol. 58:71-93, 1971) with the EGTA method of Sutherland et al. (Proc. Aust. Phys. Pharm. Soc. 11:160P, 1980) as modified by Morgan and Morgan (Fed. Proc. 41:1522, 1982). Cells loaded in this way adhere normally to culture flasks and appear normal under the microscope. They luminesce detectably in physiological salt solution and can be shown by ionophore (X537A) treatment or detergent lysis to contain large amounts of active aequorin for at least 18 hours after loading. Cells made reversibly hyperpermeable (without aequorin loading), then returned to physiological salt solution, synthesized cyclic GMP in response to agonists or Ca^{++} ionophores in an apparently normal manner (83-112% of control cells).

Experiments are in progress to examine the role of Ca^{++} in receptor-, depolarization-, and ionophore-mediated cyclic GMP formation in neuroblastoma cells. (Support: Mayo Foundation, MH27692, HL07111 and HL12186).

33.8 CALCIUM-DEPENDENT ACTION POTENTIALS OF RAT SPINAL DORSAL HORN NEURONS IN VITRO. K. Murase* and M. Randić (SPON: M.-F. Cheng). Dept. of Vet. Physiol. Pharmacol., Iowa State University, Ames, IA 50011.

The neurons of the superficial parts of the spinal dorsal horn have long resisted electrophysiological analysis due to the small size of the cells and the technical difficulties in obtaining stable intracellular recordings in vivo. We have therefore developed an in vitro rat spinal cord slice preparation in order to analyze the biophysical properties of these cells. Our initial studies have demonstrated the viability of this preparation (Miletić and Randić, Pharmacologist, 22:204, 1980) and that satisfactory intracellular recordings could be maintained for several hours (Murase and Randić, Neurosci. Abstr., 7:529, 1981).

Rats 9 to 18 days-old were used. After lumbosacral laminectomy, 300 μm thick horizontal superficial dorsal horn slice was maintained in an oxygenated Ringer solution according to the described technique (Murase et al., Brain Res., 234:170-176, 1982).

Intracellular recordings from dorsal horn neurons show that direct or orthodromic stimulation generates spike potentials followed by a brief after-hyperpolarization. Synaptic potentials were elicited by the activation of primary afferent fibers in the dorsal root. Input resistance for dorsal horn neurons ranged from 48 to 267 MΩ, and membrane time constant was in the range of 3 to 10 msec.

Dorsal horn neurons perfused with tetrodotoxin (TTX) and tetraethylammonium (TEA) frequently exhibit a slow regenerative depolarizing potential followed by a slow after-hyperpolarization in response to strong depolarizing currents. This potential is probably produced by an influx of Ca^{++}, because it is blocked by low [Ca^{++}], Co^{++} or Mn^{++}, and enhanced by high levels of extracellular Ca^{++}. A low-threshold Ca^{++} conductance is, in addition, observed in dorsal horn neurons. This conductance appears to be activated at membrane potentials more negative than -65 mV, and reaches saturation at membrane levels about -85 mV. Addition of Ba^{++} or TEA to the perfusing medium provides a suggestive evidence for the presence of inactivation of the low-threshold Ca^{++} conductance and the lack of inactivation for the high-threshold Ca^{++} potential. Certain neuroactive peptides appear to modulate calcium-dependent action potentials in dorsal horn neurons.

These results indicate that action potentials in dorsal horn neurons are generated by voltage-dependent conductance increases to sodium and calcium ions, and in particular that two distinct types of calcium spikes are probably present in these cells. The presence of voltage-dependent Ca^{++} channels may be an important mechanism for regulating the firing behavior of dorsal horn cells.

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33.9 EXCITABILITY OF GROWTH CONES OF MULTINUCLEATE PC-12 CELLS. S.L. Huttner* and P.H. O'Lague. Biology Dept., Univ. of Calif., Los Angeles, CA 90024.

Multinucleate cells (up to 250 μm in diameter) produced by polyethylene glycol fusion of cells from the clone PC-12 have been used as a model system for studying various aspects of neuronal development, particularly those of sympathetic neurons. These large cells, similar to their parent cells, respond to nerve growth factor (NGF) by growing neurite-like processes and by expressing Na-dependent action potential mechanisms; they also exhibit Ca-dependent action potential mechanisms as well as several K-dependent mechanisms (O'Lague, PH, and Huttner, SL, PNAS 77:1701-1705). The neurite-like processes of the large cells end in growing tips which resemble the growth cones of neurons in culture and which frequently are large enough to permit stable intracellular recordings. Electrophysiological recordings from these tips have been made using a single intracellular microelectrode to pass current and to record membrane voltage responses. The cells were grown with NGF (7s, 100 ng/ml) for 5 days. In every case tested (n=25) the growth cones had resting potentials of -50 to -65 mV and depolarizing current pulses elicited only passive voltage responses. However, the presence of an excitable mechanism was demonstrated by locally blocking delayed rectification by applying TEA from the tip of a micropipette (1-10 μm in diameter). Only when the pipette tip was positioned within approximately 10 μm of the growth cone were action potentials elicited. Positioning the tip over the process or the cell body failed to give action potentials when current pulses were applied in the growth cone. The action potentials were unaffected by TTX (3 μM) or by reduced external Na (15 mM) but were reversibly diminished by lowering external Ca (0.1 mM) or by applying Cd (0.75 mM). The action potential was followed by a long-lasting afterhyperpolarization (300 msec to 1 sec in duration) which resembled the Ca-dependent K-conductance found in the soma membranes of these cells (see ref. above).

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33.10 DEVELOPMENTAL CHANGES IN ELECTRICAL EXCITABILITIY OF CULTURED XENOPUS MUSCLE CELLS. P. DeCino and Y. Kidokoro. The Salk Institute, Molecular Neurobiology Lab, San Diego, CA 92138.

Cultured muscle cells from embryos of Xenopus laevis have been studied electrophysiologically to determine what ionic channels are responsible for action potential (AP) generation during development and how the addition of neural tube cells to the cultures influence these channels. Since these are small muscle cells the maximum rate of rise of the AP (V_{max}) was considered to be proportional to the ionic current density flowing during the upstroke of the AP. Comparisons between sister cultures of different ages in culture showed that V_{max} increased with time. In seven experiments in which the muscle was cultured prior to in vivo innervation V_{max} increased an average of 8 fold between days 1-2 and days 6-8 in culture. This increase was also observed in cultured muscle from later staged (post-synaptogenesis) embryos. The resting potential (average -92 to -96 mV), specific membrane resistance (~10 KΩcm^2) and capacitance (~2 μF/cm^2) did not change throughout the observation period.

In some excitable cells the ionic component responsible for the AP upstroke often shifts from Ca^{++} to Na^+ (or vice versa) during development. However, in Xenopus muscle cells under the culture conditions used here no such shift was detected. The AP rising phase always was Na-dependent and tetrotoxin (TTX) sensitive. The sensitivity to TTX ($K_{0.5}$ 9nM) did not shift with time in culture. In muscle cells cultured alone, then the developmental change was an increase in Na current density reflecting an increase in channel density and/or a change in channel kinetics.

Addition of neural tube cells often had the effect of further increasing V_{max} (2.9 fold). This effect was was independent of actual nerve contact with the muscle cell and seemed to increase when greater amounts of neural tube cells were added to the cultures. This result suggests the involvement of a diffusable substance from neural tube cells. Whatever the factor it did not seem to work by activating acetylcholine receptors found on these muscle cells since blockage with α-bungarotoxin did not alter the effect.

33.11 THE DEVELOPMENT OF SODIUM CHANNELS DURING DIFFERENTIATION OF CHICK SKELETAL MUSCLE IN CULTURE. J. Baumgold, J. B. Parent* and I. Spector*. Lab. of Neurobiol., NIMH, Bethesda, MD 20205, Howard Univ. Cancer Ctr., Washington, DC 20060, Lab. of Biochem. Genetics NIH, Bethesda, MD 20205.

The development of sodium channels during differentiation of chick skeletal muscle in culture was followed at three different levels of cellular organization: 1) ^{3}H-saxitoxin (^{3}H-STX) and ^{125}I-scorpion toxin (^{125}I-ScTX) binding were used to monitor the appearance and elaboration of two different binding sites on the sodium channel; 2) ^{22}Na-uptake studies were performed in order to assess the capacity of these developing channels to function in mediating ^{22}Na-uptake when stimulated with various toxins, and 3) the electrophysiological responses of these cultures were recorded in order to assess the functionality of these channels. When the development of the sodium channel was assayed using binding studies, we found that, whereas ^{3}H-STX binding developed gradually after cell fusion and reached a maximal value (58 fmol/mg protein) after 8 days in culture, the ^{125}I-ScTX binding developed rapidly during and after cell fusion to reach a maximal value by 4 days in culture. Two different rates of development were also found with the ^{22}Na-uptake studies. When ^{22}Na-uptake was stimulated using batrachotoxin (200 nM), the initial rates of ^{22}Na-uptake increased gradually after fusion to reach a maximal value of 12 nmoles of Na/min/culture by 8 days in culture. When the ^{22}Na-uptake was stimulated by both scorpion toxin (300 nM) and batrachotoxin (200 nM), however, the cultures exhibited a rapid increase in their initial rate of ^{22}Na-uptake after fusion. Sodium dependent action potentials could first be detected in 4 day old cultures. However, sodium dependent action potentials could be induced in 3 day old cultures within minutes after addition of 200 nM ScTX. In the presence of ScTX the rate of rise and amplitude of the sodium action potential develops earlier and reaches a higher maximal value than in the absence of this toxin.

Based on this data, we propose the following model for the development of sodium channels. We hypothesize that this intrinsic membrane protein gets incorporated into the membrane in an immature form, capably of binding ^{125}I-ScTX but not ^{3}H-STX. This immature channel is electrophysiologically non-functional. In the course of maturation, we predict that this protein undergoes a post-translational modification or aggregation which renders it electrophysiologically functional and capable of also binding ^{3}H-STX. We further predict that this maturation process can be mimicked, on a more rapid time scale, by the addition of ScTX. Thus, the addition of ScTX to immature cells induces the post-translational modification or aggregation to occur in minutes, rather than in days.

33.12 EFFECT OF COCULTURE AND in vitro (RE)INNERVATION ON THE ELECTRICAL MEMBRANE PROPERTIES OF RAT MYOTUBES. B.A. Suarez-Isla*, J.M. Thompson and S.I. Rapoport (Spon: Anne E. Schaffner) Laboratory of Neurosciences, NIA, NIH, Baltimore, Maryland 21224.

Intracellular recording techniques have shown that neurons dissociated from 8-day chick embryo neural retina form transient synapses with rat muscle cells in culture that begin to be suppressed within 48 h of coculture (Ruffolo et al., PNAS, 75, 2281, 1978). On the contrary, dissociated spinal cord neurons (SCN) from the same source form stable synapses that generate spontaneous m.e.p.p.s. even after 2 weeks in coculture. We report here the effects elicited by 24 to 72 h coculture with chick embryo neurons on the electrical membrane properties of cultured muscle cells. All differences reported refer to paired sets of control(C) and coculture(CC) dishes in which 8 to 10 unbranched, spindle-shape myotubes (6 to 14 days old) were tested (values given as mean + S.D.). We found that only neurons that form stable synapses (SCN) induced a significant decrease ($P<0.01$) in the incidence of slow hyperpolarizing after potentials (slow HAPs; τ=90-200 ms at 37°C; RMP>-45 mV) following an overshooting action potential evoked by anode break excitation. The percentage of cells showing a distinct slow HAP decreased from 86.9 + 12.8 (C; 87/100 cells) to 52.2 + 20.4 (CC; 42/79 cells). This result was obtained from 17 matched pairs of dishes coming from 6 primary dissociations. Comparison of frequency distribution histograms of specific membrane resistance (R_m; bin size=200Ω.cm^2) of cells from all dishes with slow HAPs and cells without them, indicated a significant shift towards lower R_m values ($P<0.01$; Kolmogorov-Smirnov test) in cells devoid of HAPs (mean cells with HAPs=918Ω.cm^2; N=98; without HAPs=608Ω.cm^2; N=86). When C and CC dishes were separately analyzed the shift to lower R_m was also evident, the cells without HAPs in the CC dishes being responsible for most of the shift. However, no significant differences in HAP incidence or R_m distribution were found among CC cells with or without spontaneous synaptic activity. A small but significant ($P<0.001$) decrease in RMP occurred 72 h after coculture (C: -51.0+8.2 mV; N=167. CC: -45.1 +9.7 mV; N and subsequent to a significant decrease in slow HAP incidence (24 h). All these effects could be induced by SCN conditioned medium. However, similar periods of coculture with neurons from chick retina elicited none of these changes.

The slow HAP and its associated Ca^{2+}-dependent K^+-conductance (Barrett et al., Dev. Biol., 82, 258, 1981) seem to be responsible in part for maintaining spontaneous contractile activity before innervation in vivo. This mechanism is partially suppressed during (re)innervation in vitro by neurons that form stable synapses but not by those that form only transient synaptic contacts. These findings support the view that the processes of synapse selection and stabilization in vitro are contemporary to the induction of specific changes in the electrical membrane properties of cultures muscle cells.

33.13 THE IONIC BASIS OF THE ACTION POTENTIAL IN NERVE TERMINALS OF A MOTOR NEURON NERVE NET. Peter A. V. Anderson and Walter E. Schwab*, Whitney Marine Lab and Dept. of Physiology, University of Florida, Gainesville, FL 32611 and Dept. of Biology, Virginia Polytechnic Institute and State University, Blacksburgh, VA 24061.

The motor neuron nerve net of the jellyfish Cyanea capillata is a diffuse network of small (cell body diameters 10-20 μm, axon diameter 5 μm) bipolar neurons. Electronmicroscopy has shown that these neurons are connected by symmetrical chemical synapses; that is, each terminal contains an active zone and an accumulation of vesicles with the active zones of the two terminals directly apposing one another. This organization implies that transmission across the synapse is non-polarized and chemical and this has been confirmed physiologically.

The dimensions of the cells are such that we estimate that a given cell body is within a fraction of a space constant of a synapse. Consequently, an electrode in the soma will accurately monitor events in the pre- or post-synaptic terminals of nearby synapses, thereby allowing the physiology of the synapses to be examined in detail.

The neurons have a negative resting potential (-58mV) and produce very complex and variable positive-going spikes. However, when Cd^{++} and Ni^{++} are added to the sea water, or if cells are bathed in high Mg^{++}, low Ca^{++} artificial sea water, the complexity and variability is lost, leaving a clean, fast, overshooting action potential. At the same time, synaptic transmission is blocked. Somewhat similar action potentials can be recorded in sea water when neurons are stimulated extracellularly with paired stimuli at intervals less than the relative refractory period of the cells. The conclusion from these observations is that the complexity and variability of the spike is the result of the superimposition of synaptic potentials and a single small calcium dependent event on the action potential. The action potential itself is sodium dependent but TTX insensitive, and repolarization is the result of two pharmacologically distinct mechanisms. A delayed, Ca^{++} activated K^+ efflux which produces an after-hyperpolarization and a tetraethylammonium and 4-aminopyridine sensitive, presumably voltage activated, early potassium efflux.

These various properties of the neurons are very conventional despite the phylogenetic primitiveness of the animal. Therefore, since it is possible to impale the pre- and post-synaptic terminals of the synapses, we have a preparation with which to examine the physiology of excitatory chemical synapses in both the pre- and post-synaptic terminals.

33.14 **ORIGIN OF THE DEPOLARIZING AFTER-POTENTIAL IN APLYSIA CELL R15.** **William B. Adams and Irwin B. Levitan. Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.**

In Aplysia cell R15, and in many other spontaneously active cells, individual action potentials are followed by a depolarizing after-potential (DAP). In those cells that can display either beating or bursting activity, it is the presence or absence of the DAP that determines the form of activity; bursting cells have a DAP, beating cells do not. When R15 is voltage clamped, brief (10 msec) depolarizing pulses activate a tail current that consists of three components: a rapidly decaying current from the Na^+, Ca^{++}, and K^+ channels that are activated during the pulse; a slower inward current with kinetics matching those of the DAP; a very slow hyperpolarizing current.

Several lines of evidence suggest that the inward current that produces the DAP arises from passive spread of current back into the soma from an action potential that is propagating down the axon away from the soma: (1) Activation occurs over a very narrow range of depolarizing pulse potentials. (2) Under normal conditions, activation of the inward current requires both Na^+ and Ca^{++} in the bathing medium, reflecting the mixed Na^+/Ca^{++} action potential in R15. If K^+ channels are blocked by Cs^+, 4-AP, and TEA, either Na^+ or Ca^{++} suffices. (3) With K^+ channels blocked, it is possible to prevent activation of the inward current by following the depolarizing pulse with a brief hyperpolarizing pulse. The necessary amplitude of the hyperpolarizing pulse depends linearly on the amplitude of the depolarizing pulse and not on the amount of activation of the inward current. (4) DAPs are not found in R15s in which the axons have been ligated.

33.15 SLOW ACTION POTENTIAL IN THE GIANT MOTONEURON OF CRAYFISH : CONDITIONS FOR TRIGGERING, IONIC MECHANISM AND LOCALIZATION. G. Czternasty*, R.T. Kado* and J. Bruner, Lab. de Neurobiologie, Université de Picardie, 80039 Amiens and Lab. de Neurobiologie cellulaire, C.N.R.S., 91190 Gif-sur-Yvette, France.

The slow action potential (SAP) in the giant motoneuron (MoG) somata, described previously (Czternasty & Bruner, Neurosci. Lett., suppl. 7, S.379, 1981) was further studied in the present work under current and voltage clamp. The SAP (threshold at -19 mV ± 2, N = 4) is voltage and time dependent on conditioning depolarization of several seconds (to -50 mV). The constant outward conditioning current produces a slowly increasing depolarization which reaches a plateau. Hyperpolarizing test current pulses during the conditioning depolarization show that the input resistance increases 2 to 3 times. Depolarizing test pulses show a decrementing depolarization. This decrement disappears with conditioning time and the depolarizing pulse produces a SAP going to about +10 mV. Under voltage clamp from holding at -70 mV to holding -50 mV the outward current diminishes with time to about a third of its initial value. I-V curves obtained before, during and after the conditioning period show markedly decreased slopes after conditioning. A slow inward current ($I_{in.s.}$) also appears after conditioning. The SAP and $I_{in.s.}$ persist in Na-free or TTX (10^{-6} M) solution, are blocked in Co^{++} (10 mM) or Mn^{++} (2,5 mM) solution and cannot be triggered in zero Ca^{++}. On the other hand $I_{in.s.}$ become progressively larger with increasing $[Ca^{++}]$ out. Finally, net $I_{in.s.}$ did not reverse for depolarizations as high as +80 mV. Simultaneous recording of the local membrane current of the soma with a pair of extracellular electrodes indicates a somatic localization for these currents.

These results indicate that the SAP is due to somatic, voltage dependent calcium channels. The channels can produce a SAP or $I_{in.s.}$ in voltage clamp from -70 mV if the cell is injected with TEA. In non injected cells the conditioning depolarization is necessary to produce the same effects. A tentative interpretation of these results would be that a voltage dependent inactivation of the delayed rectification channels exists in the MoG membrane.

33.16 MAMMALIAN CEREBRAL AXONS: DIFFERENTIAL EFFECTS OF 4-AMINOPYRIDINE ON SLOW AND FAST FIBERS. R. J. Preston*, S. G. Waxman and E. C. Lampley*. Dept. of Neurology, Stanford Sch. of Med. and VA Med. Ctr., Palo Alto, CA 94304.

Studies of mammalian peripheral and spinal myelinated axons suggest that they exhibit a relatively small response to potassium conductance (g_k) blocking agents that readily alter action potentials in nonmyelinated fibers. We have investigated the effects of the g_k blocker 4-aminopyridine (4-AP) on conduction in mammalian cerebral axons.

Wistar rats (250-300 gm) were anesthetized with Ketamine plus Xylazine to allow recording of the compound action potential (CAP) in the anterior corpus callosum at distances of 1 to 3 mm from stimulation electrodes. Prior to 4-AP application the callosal CAP was characterized by two closely apposed short duration (0.5-1.5 msec) negative waves. These had onset latencies suggesting near maximal conduction velocities of 8.0 and 2.2 m/sec respectively, for two discrete groups of constituent fibers. The application of 4-AP typically caused reversible increase in the duration of the late wave without marked effect on the early negativity. This result was achieved with either of two procedures. In one, recording and stimulation electrodes were stereotaxically positioned and the tip (30-50 μm) of an injection pipette was positioned along the fiber path between them and within 200-800 μm of the recording electrode. From 0.1 to 0.3 μl of 0.2-0.5 mM 4-AP was ejected over a period of 60 sec. In the other procedure, cortical tissue was removed by suction to permit placement of stimulating and recording electrodes under visual control and to allow superfusion of callosal fibers with normal or 4-AP solutions warmed to 37° C.

We interpret these experiments as indicating that the potassium conductance blocking action of 4-AP rapidly alters action potential mechanisms of slowly conducting, presumably non-myelinated callosal axons, but does not affect the action potential of more rapidly conducting myelinated fibers. These results suggest that the finding that peripheral and spinal myelinated axons may lack significant nodal potassium conductance may be extended to mammalian cerebral myelinated axons.
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34 SYMPOSIUM. THE ASSEMBLY OF SOMATOTOPIC MAPS IN THE CENTRAL NERVOUS SYSTEM: A CARTOGRAPHER'S DELIGHT. R.K. Murphey, SUNY Albany, NY (Co-chairman) and H.P. Killackey, U.C. Irvine (Co-chairman); M. Constantine-Paton, Princeton; E. Macagno, Columbia.

The existence of highly ordered afferent projections has been a topic of interest to neuroscientists and developmental biologists since the turn of the century. Numerous hypotheses have been put forward to account for the precision of this mapping. Most hypotheses assume that the information necessary for the assembly of such maps exists as cues for the growth and maintenance of neural processes. Most of these hypothetical cues can be grouped under one of three headings: spatiotemporal cues, chemoaffinity cues and competitive interactions. The participants have each focussed their attention on a sensory system in a different species. Dr. Macagno will discuss aspects of the development of the compound eye of Daphnia and other invertebrates, Dr. Killackey the vibrissal system of the rat, Dr. Constantine-Paton the retino-tectal system of the frog and Dr. Murphey the cercal afferent system of the cricket. Results from each of these species will be discussed in the context of possible mechanisms for the assembly of somatotopic maps. We will highlight similarities and differences among the various species and attempt to assess the likely role of previously proposed cues in guiding neurons to their proper targets.

There is considerable controversy in the field and the various biases of the participants represent most of the possible positions which could be adopted on this topic. As a result we expect some lively exchanges among the participants and the audience.

35 SYMPOSIUM. SYMPATHETIC GANGLIA AS MODELS FOR SYNAPTIC TRANSMITTER ACTIONS. B. Libet, Univ. of California, San Francisco (Chairman); J.H. Ashe, Univ. of California, Riverside; L. Jan, Univ. of California, San Francisco; S. Konishi*, Tokyo Medical and Dental University.

Views of synaptic transmitter actions in sympathetic ganglia have progressed from that of a cholinergic-nicotinic one, related to that at neuromuscular junctions, to include a variety of novel actions mediated by ACh, catecholamines and peptides. Since these actions are analyzable in relatively simple and definable synaptic pathways they provide models for as yet unspecified synaptic actions in related transmitter systems in the brain.

Various lines of evidence have indicated that a second transmitter (a catecholamine), released by an intraganglionic interneuronal structure, is the direct mediator for the slow IPSP's. John Ashe will discuss this proposition and the more recent evidence on it.

The slow EPSP is a direct muscarinic response to ACh. Ben Libet will discuss the potential contributions to it of a cyclic GMP-mediated component with no change in membrane conductance, and one due to muscarinic closure of the "M"(K^+) channels with a decrease in conductance.

The long-term potentiation by one transmitter (dopamine, DA), of the s-EPSP responses to another transmitter (ACh), provides a unique model for a heterosynaptic modulatory interaction. Libet will discuss features of this action and evidence specifying the modulatory DA receptor as one coupled to adenylate cyclase.

An even later s-EPSP, with durations of many minutes and mediated non-cholinergically, has also been demonstrated in frog and more recently in mammalian ganglia. Lily Jan will discuss the identification of the peptide LH-RH as the probable transmitter in frog ganglia, features of release and postsynaptic action of LH-RH, and possible roles of other peptides.

Enkephalin is one of a number of peptides found in mammalian ganglia. Shiro Konishi will discuss evidence indicating that presynaptic inhibition can be produced in these ganglia by orthodromic (preganglionic) input, and that intraganglionic enkephalin appears to be the mediating transmitter acting on the presynaptic terminals.

36.1 MONOCLONAL ANTIBODIES THAT RECOGNIZE SPECIFIC RETINA CELL TYPES, AFFECT SYNAPTIC FUNCTION, OR INHIBIT CELL ADHESION. Gerald B. Grunwald, G. David Trisler*, Haruhiro Higashida*, Paul Darveniza* and Marshall Nirenberg. Laboratory of Biochemical Genetics, NHLBI, NIH, Bethesda, MD 20205.

We are using embryonic chick neural retina as a model system for studies on synapse formation and neuron differentiation. Monoclonal antibodies were generated by fusing P3X63 Ag8 myeloma cells with spleen cells from mice immunized with 14 day chick embryo retina cells or mouse or rat embryo retina-neuroblastoma hybrid cells.

Antigen distribution in retina was determined with frozen sections of retina and fluorescein-conjugated IgG or peroxidase-conjugated IgG directed against mouse immunoglobulin. Four antibodies were found that bind to photoreceptor outer segments but not to other cells in retina, three bind to photoreceptor soma, three bind to antigens in the outer synaptic layer of retina, two bind to antigens restricted to cell soma in the inner nuclear layer, one binds to an antigen localized in discrete clusters in the inner synaptic layer, two bind to antigens associated with ganglion neuron soma, and six bind to Muller cells. Other patterns of antigen distribution were detected with 22 other antibodies. For example, 7 antibodies bind to the outer limiting membrane of retina, the outer synaptic layer, and localized clusters of antigen associated with amacrine and ganglion neuron soma.

Four patterns of antibody binding were observed with 7 day chick embryo retina: antibodies bind to cells located at the outer margin of retina, the inner margin, the inner and outer margins, or to cells uniformly distributed in retina. These patterns of antibody binding change between the 7th and 14th day of development. Many antigens that were not detected in 7 day embryo retina were expressed by the 14th day of development.

Preliminary data indicate that six of the antibodies to chick retina inhibit intercellular adhesion. The frequency of myotube miniature end plate potentials between NBr10-A neuroblastoma hybrid cells and striated muscle cells was increased 200-400% by four antibodies, and decreased greater than 50% by two antibodies directed against retina-neuroblastoma hybrid cells. Thus, monoclonal antibodies were obtained that recognize specific cell types in the retina, affect transynaptic communication or inhibit intercellular adhesion.

36.2 A TRYPSIN SENSITIVE ACETYLCHOLINE SECRETION REACTION IN CHICK EMBRYO RETINA CELLS - POSSIBLE REQUIREMENT FOR SYNAPSE FORMATION. R. Ray, J.M. Thompson, and M. Nirenberg. Lab. Biochem. Genetics, NHLBI, NIH, Bethesda, MD 20205.

Some neurons dissociated from chick embryo retina with trypsin formed synapses with cultured striated muscle cells after incubation with muscle cells for >75 min; whereas, few synapses were formed during the initial 75 min of incubation (Puro, D. G., et al. (1977) Proc. Natl. Acad. Sci. USA, 74, 4977). To see whether dissociation of cells with trypsin decreases the ability of the cells to secrete acetylcholine (ACh), we determined the effect of trypsin on ACh secretion by 12 day chick embryo retina cells perfused in vitro with medium containing 5.4 or 81 mM K^+. Retina cells were incubated with [^{3}H]-choline, washed, and [^{3}H]-ACh secreted into the medium was determined. Trypsin had no effect on the basal rate of [^{3}H]-ACh secretion in medium containing 5.4 mM K^+, but [^{3}H]-ACh secretion due to depolarization by 81 mM K^+ was decreased approximately 50%. Stimulus-dependent ACh secretion is known to require Ca^{2+} ions; however, no effect of trypsin was detected on $^{45}Ca^{2+}$ uptake by retina cells at 5.4 or 80 mM K^+. However, the increase in $^{45}Ca^{2+}$ uptake by NBr10-A neuroblastoma-buffalo rat liver hybrid cells due to 80 mM K^+ was inhibited approximately 50% by trypsin. NBr10-A cells possess voltage-sensitive Ca^{2+} channels, secrete ACh, and form synapses with striated muscle cells. The apparent insensitivity of voltage-dependent Ca^{2+} channels of chick embryo retina cells to trypsin under the conditions tested does not preclude the possibility that these Ca^{2+} channels may be sensitive to trypsin under other conditions. The demonstration that trypsin inhibits K^+-dependent ACh secretion, but not $^{45}Ca^{2+}$ uptake by chick embryo retina cells suggests that a component or components required for ACh secretion other than voltage-sensitive Ca^{2+} channels may be sensitive to trypsin and may be required for synapse formation.

36.3 FUNCTIONAL AND BIOCHEMICAL DIFFERENTIATION OF MOTOR NERVE TERMINALS FORMED IN THE ABSENCE OF MUSCLE FIBERS. M.A. Glicksman* and J.R. Sanes. (SPON: M. Willard) Department of Physiology Washington University School of Medicine, St. Louis, MO 63110

During reinnervation of adult vertebrate skeletal muscle, axons regenerate and form new, functional nerve terminals at original synaptic sites on denervated muscle fibers. When muscle is damaged as well as denervated, myofibers degenerate but their sheaths of basal lamina (BL) persist. Axons regenerate to contact original synaptic sites on the BL sheaths and there acquire morphological characteristics of nerve terminals such as synaptic vesicles and active zones; BL regulates this differentiation (J. Cell Biol. 78:176,1978). We show here that these nerve terminals, formed in the absence of the postsynaptic cell, also differentiate physiologically and biochemically.

Both cutaneous pectoris muscles of the frog were denervated and damaged, to obtain nerve- and muscle-free BL sheaths. Frogs were x-irradiated to prevent myofiber regeneration. Muscles were examined 3 weeks later, when reinnervation was maximal. To study the functional capacity of nerve terminals, paired muscles were bathed in horseradish peroxidase (HRP) while the nerve on one side was stimulated (10 Hz for 1h with 30 sec rest every 5 min), then reacted for HRP and prepared for electron microscopy. The appearance of HRP in vesicles indicates endocytosis and, by implication, vesicle recycling (J. Cell Biol. 54:30,1972; 57:315,1973). 76±14% (SE,n=4 muscles) of the terminals on the stimulated side contained >1 labeled vesicle compared to only 9±5% on the unstimulated side. Biochemical differentiation was assayed immunocytochemically with an antiserum to Torpedo electroplax synaptic vesicles that selectively stains nerve terminals in adult muscle (Nature 280: 403, 1979). Cryostat sections were incubated with antiserum and fluorescein-conjugated second antibody; synaptic sites were identified with a histochemical stain for acetylcholinesterase (which is in BL and survives muscle damage). Antibody-stained structures were present at (although not confined to) synaptic sites. We conclude that axons can acquire morphological, immunochemical and physiological properties of normal nerve terminals in the absence of the postsynaptic cell, and that BL triggers some step(s) in this process. (Supported by MDA and NSF.)

36.4 DIFFERENTIATION OF BASAL LAMINA IS AN EARLY EVENT IN THE DEVELOPMENT OF THE NEUROMUSCULAR JUNCTION IN VIVO. A.Y. Chiu and J.R. Sanes, Department of Physiology, Washington University School of Medicine, St. Louis, MO 63110

Muscle fiber basal lamina (BL) survives injury to nerve and muscle, and regulates differentiation of both pre- and postsynaptic elements when neuromuscular junctions reform (J. Cell Biol. 78:176,1978; 82:412,1979; Glicksman and Sanes, this vol.). If BL plays comparable roles during development, its differentiation and that of nerve and muscle must be interdependent. As a first step in understanding these interactions, we have begun to correlate appearance of individual BL components with known events in synaptogenesis. In the rat, acetylcholine receptors (AChRs) and acetylcholinesterase become concentrated at synaptic sites on embryonic day 16 (E16; birth = E22), whereas other steps in neuromuscular maturation, such as elimination of polyneuronal innervation and formation of junctional folds, occur postnatally. We show here that differentiation of muscle fiber BL into immunocytochemically distinct synaptic and extrasynaptic regions begins by E16.

Antisera distinguish three classes of components associated with BL -- those concentrated at synapses, a second class concentrated extrasynaptically and a third shared by synaptic and extrasynaptic BL (J. Cell Biol. 83:357,1979; 93:442,1982). We used one antiserum of each class and also generated a monoclonal antibody that recognizes a synaptic antigen previously defined with serum. Cryostat sections of embryonic rat intercostal muscles were incubated with anti-BL and fluorescein-second antibody; rhodamine-α-bungarotoxin, which binds to AChRs, was added to identify synaptic sites. Shared and extrasynaptic antigens are present over parts of the myotube surface by E15. On E16, when bungarotoxin first identifies synaptic sites, the synaptic antigen is concentrated at some of them. By E17 the synaptic antigen is concentrated at and the extrasynaptic antigen excluded from many synapses; shared antigens are present both synaptically and extrasynaptically. The small set of antisera used so far does not reveal (but certainly does not rule out) qualitative changes in BL during later stages of development. Synapse-specific staining of embryos and adults with the monoclonal antibody shows that the synaptic antigen present in embryos is closely related to that in adults. Our results show that the BL is already divided into chemically distinguishable synaptic and extrasynaptic domains at an early stage in neuromuscular development in vivo and thus could, in principle, be involved in regulating later events. (Supported by MDA and NSF.)

36.5 ACTIVITY AND NEURAL EXTRACT REGULATE ACCUMULATION OF BASAL LAMINA BY CULTURED MYOTUBES. J.R. Sanes, D.H. Feldman and J.C. Lawrence, Jr.*, Depts. of Physiology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110

Skeletal muscle fibers are ensheathed by a basal lamina (BL), which extends through the synaptic cleft and plays important roles in neuromuscular regeneration and function (J. Cell Biol. 78:176,1978; 82:412,1979). Antigens associated with BL are differentially distributed: some ("synaptic") are concentrated at synapses, some ("extrasynaptic") are excluded from synapses, and some ("shared") are present in both synaptic and extrasynaptic BL (J. Cell Biol. 83:357,1979; 93:442,1982). It is likely that nerve and muscle interact during development to produce this pattern. Two ways in which nerves can influence muscle fibers are by causing muscle activity and by releasing soluble factors. We used myotubes cultured from 19d rat embryos to study the effects of activity and a neural extract on accumulation of BL. Tetrodotoxin (TTX) or lidocaine were used to paralyze the normally spontaneously active myotubes. A saline extract of adult rat brain was the source of soluble neural factors. BL was studied by LM and EM immunocytochemistry and by ^{125}I-antibody binding, using antisera to "synaptic" and "shared" BL components.

Spontaneously active myotubes accumulate BL over much of their surface. Shared antigens are present throughout the BL while synaptic antigens are concentrated in small patches, often associated with clusters of acetylcholine receptors (AChRs; visualized with rhodamine-α-bungarotoxin). Paralyzed myotubes accumulate less shared antigen-rich BL than active myotubes. New BL forms if TTX is removed from cultures, and BL is lost if active myotubes are paralyzed. However, synaptic antigen-rich patches are not lost, but actually increase in number when myotubes are paralyzed. Thus, activity stimulates BL accumulation overall, by a selective effect on shared antigens.

Application of brain extract also enhances BL accumulation. However extract, unlike activity, greatly increases the number of synaptic antigen-rich patches. Extract also increases the number of AChRs and of AChR-rich clusters; new synaptic BL- and AChR-rich patches generally coincide. Thus, activity and neural factors both increase BL accumulation, but in different ways. Our results suggest that nerves could regulate accumulation of BL by myotubes in two ways--shared components being stimulated primarily by nerve-evoked activity, and synaptic components by soluble factors released from the nerve. (Supported by MDA and NSF.)

36.6 THE INVOLVEMENT OF CALCIUM IN THE FORMATION OF ACH RECEPTOR CLUSTERS. H. Benjamin Peng* (SPON: C. Anderson). Dept. of Anatomy, Univ. of Illinois College of Medicine, Chicago, Illinois 60612.

Recently we have shown that the clustering of acetylcholine receptors (AChRs) can be induced in cultured Xenopus muscle cells by polypeptide-coated latex beads (Nature 292:831). A complete set of structural specializations characteristic of the postsynaptic membrane also develops at the bead-muscle contacts. Thus the bead-muscle coculture can be used as a model for understanding the cellular processes involved in the formation of AChR clusters during innervation.

The induction of AChR clustering by the latex beads can occur in a medium consisting only of inorganic ions. We hypothesize that the beads may cause a local change in the permeability to certain ion(s), thus triggering the machinery for the AChR clustering. In particular, the involvement of calcium was tested through the use of calcium antagonists. The formation of the clusters was induced by polyornithine-coated latex beads and the clusters were assayed by labeling the cells with tetramethyl rhodamine-conjugated α-bungarotoxin followed by fluorescence and phase microscopy after a 2 hr to overnight bead-muscle coculture. The mean percentage of beads which were associated with AChR clusters was used as an index. In calcium-free medium containing 0.4mM EGTA, the formation of clusters was inhibited. The beads also failed to induce clustering in the presence of 5mM $CoCl_2$, 5mM $NiCl_2$, 0.3mM verapamil or 0.3mM D-600. In the control, 60-90% of the beads would induce clusters, whereas in the presence of these calcium antagonists only less than 10% of the beads were associated with clusters. This suppression is reversible - if the antagonists were removed following a 2 hr incubation, the formation of clusters returned to 70-100% of the control level. Existent clusters were much less affected by the antagonists-if the clusters were first induced by the beads followed by the addition of antagonists, the association of clusters with the beads was still seen. Two other agents, 5mM $MnCl_2$ and 0.5mM nifedipine, were found to be ineffective in suppressing the clustering.

The effect of these agents in interfering with the entry of calcium into the cells is well known. Our results thus suggest that the induction of AChR clustering by the latex beads is mediated by a Ca^{2+}- regulated process. Since the clusters at the bead-muscle contacts are highly localized, this process must likewise be activated in a highly localized manner. Thus we propose that a local rise in intracellular calcium concentration may trigger the formation of AChR clusters. (Supported by NIH grant NS 16259 and MDA)

36.7 CHARACTERIZATION OF IDENTIFIED MOTONEURONS IN VITRO. R.J. O'Brien*, L.W. Role and G.D. Fischbach. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Using a technique similar to that of Okun and McPheeters (Soc. Neurosci. 6:733) we have identified living motoneurons amidst a heterogeneous population of chick spinal cord cells in culture. A high concentration of both Lucifer Yellow V.S. and Wheat Germ Agglutinin-Lucifer Yellow was injected into the legs of 5-day chick embryos. After 16 hrs of retrograde transport cells were dissociated and plated on glass coverslips. Frozen sections of chick spinal cord showed that the label was confined to the anterior horn. Cell counts on the stage of a microscope equipped with inverted fluorescence optics showed that 60% of the total lumbar motor column can be identified with this technique. This figure amounts to about 3% of the total cells in our culture.

Up to 7 days in culture the number of fluorescently labeled motoneurons decreased with a half life of 2 days when grown in media containing 5% chick embryo extract (CEE) on collagen or on lysed fibroblasts. Growth in 0% CEE on lysed fibroblasts was indistinguishable from that in 5%; these neurons had extensive processes and appeared quite healthy at 7 days in vitro. Motoneurons grown on collagen with 0% CEE showed nearly a 4-fold decrease in number at 3 days and an 8-fold decrease by 5 days in culture when compared to the three other growth conditions. Comparison with other cell types indicates that the survival effect of lysed fibroblasts in 0% CEE may be specific for motoneurons.

This system presents an opportunity for studying whether cells of similar origin (i.e., spinal cord) have an equal capacity to induce clusters of acetylcholine receptors on myotubes. Using Lucifer Yellow CH injection and rhodamine α-bungarotoxin staining we found that 95% of the motoneurons had neurites associated with rhodamine patches, as compared to 19% for the nonmotoneurons. When expressed in terms of the number of receptor clusters per area of nerve-muscle contact, motoneurons had 16 times more neurite-associated receptor clusters than nonmotoneurons (1.1/100 μ vs. .067/100 μ).

Intracellular microelectrode and patch clamp recordings show that identified motoneurons are electrically excitable and that they can form chemical synpases with muscle. Dye-coupling was investigated by intracellular injection of Lucifer Yellow. Between days 1 and 3 in vitro 24% of the motoneurons were dye-coupled to another neuron as opposed to 5% of the nonmotoneurons. By 6 days in vitro only 6% of the motoneurons were coupled to other neurons. We have also observed 3 examples of dye-coupling between motoneurons and flat cells, and one example of dye-coupling between a motoneuron and a myotube. Chemosensitivity and interneuron synaptic studies are underway.

36.8 TRANSMITTER RELEASE AND RECEPTOR AGGREGATION AT CILIARY NEURON-MUSCLE SYNAPSES. L.W. Role, R.I. Hume and G.D. Fischbach. Dept. Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

The chick ciliary ganglion contains cholinergic neurons which innervate muscle in vivo. In culture ~70% of the ciliary neurons form functional synapses on muscle by 1-2 weeks (Nishi & Berg PNAS 74:5171, 1977). We have exploited this high degree of connectivity to examine the early events in synaptic transmission and the distribution of acetylcholine receptor (AChR) clusters along the full extent of the neurite arborization.

At 12-24 hr after plating, intracellular stimulation and recording demonstrated short latency (2-5 msec) rapidly rising (2.5-10 msec time to peak) postsynaptic potentials (psp) in 60% of the nerve-muscle pairs tested (n=66). Amplitude histograms obtained on 14 of 16 pairs tested are consistent with a quantal release mechanism at these early synapses.

The earliest contacts rapidly fatigue so that long series of psp's could not be evoked. However, it is clear that synapses can form within 2 h. Between 1-12 h after plating 28 of 97 nerve muscle pairs tested were synaptically connected. Most of the psp's were rapidly rising discrete responses. However, on occasion long latency (20-50 msec), very slow rising (50 msec time to peak), long duration (>100 msec) nonfluctuating responses were recorded. Out of 182 nerve-muscle pairs tested with intracellular recording, 2 examples of electrical coupling were observed. In one of these cases injection of Lucifer Yellow revealed that the cells were also dye-coupled.

The relationship between the neuronal arborization and distribution of AChR clusters in neuron muscle cocultures was studied by injecting neurons with Lucifer Yellow and labeling receptors with rhodamine α-bungarotoxin (RBTX). Within 24-36 h an individual neuron can induce as many as 30 nerve-associated RBTX patches; as many as 10 RBTX patches may be associated with a given neurite on the same muscle. There were 2.3±0.3 RBTX patches/100 μ nerve muscle contact (24-36 h). This is similar to the value obtained when identified spinal motoneurons are studied in the same manner (see O'Brien et al., this volume). Twenty-five percent of the neurite endings, most with typical growth cone morphology, have associated RBTX patches (12-36 h).

We have observed rapid rise psp's in the absence of subneural RBTX patches. We have also observed quantal psp fluctuations with only 1 subneural RBTX patch. Thus, fluctuations in amplitude of the psp's do not necessarily correlate with the number of synapses as indicated by AChR clusters. The precise distribution of subneural AChR clusters and parameters of transmitter release is being investigated further.

36.9 RISE TIMES OF SYNAPTIC CURRENTS AT THE DEVELOPING NEUROMUSCULAR JUNCTION. R. W. Kullberg and H. Kasprzak*. Biology Dept., Univ. Alaska, Anchorage, AK 99508.

The rise times of miniature endplate currents (MEPCs) decrease from about 2 msec to less than .5 msec during development of the myotomal neuromuscular junction of Xenopus laevis (Kullberg, R.W., Mikelberg, F.S. & Cohen, M.W., Develop. Biol., 75:255, 1980). This change appears to be due to two developmental processes: an increase in acetylcholinesterase (AChE) activity and a decrease in acetylcholine receptor (AChR) channel open time. At the newly formed synapse, little or no AChE activity can be detected, whereas at the mature synapse, application of anticholinesterase prolongs synaptic currents and Karnovsky stain shows abundant esterase at the synaptic regions (Kullberg et al, ibid.). During the same period of time that AChE activity is increasing, the open time of AChR channels decreases from an average of 3.0 msec to 0.7 msec (Kullberg, R.W., Brehm, P. & Steinbach, J.H., Nature, 289:411, 1981; Kullberg, R.W. & Steinbach, J.H., unpublished). The effects of these two processes on the rise times of synaptic currents were examined by blocking AChE activity at the mature synapse and substituting embryonic AChR channels in place of mature ones by a mathematical procedure. Under control conditions, the mean rise time of externally recorded MEPCs at the mature myotomal synapse was 0.4 $\pm$ 0.1 msec (mean $\pm$ s.d., n = 26 recording sites). Application of 3 mM methanesulfonyl fluoride abolished AChE activity and lengthened rise times to 0.7 $\pm$ 0.2 msec (n = 17 sites). Embryonic channels were then mathematically substituted in place of mature ones, and the MEPC rise times were further prolonged to embryonic values. Channel substitution was done by treating each MEPC as a convolution between a driving function, Y(t), representing the frequency of channel opening, and an exponential function, exp(-t/tau), describing the rate of channel closing (tau = mean channel open time). The shape of Y(t) was calculated from single MEPCs according to the equation: $Y(t) = C(dV(t)/dt + V(t)/tau)$, where V(t) = MEPC voltage, tau = 0.7 msec and C = arbitrary constant with units of channels/mV. The calculated Y(t) from each mature MEPC was then convolved with exp(-t/3msec) to produce a corresponding theoretical embryonic MEPC. This procedure was carried out on 390 MEPCs sampled at 17 recording sites. The mean rise time was 2.2 $\pm$ 0.6 msec, which compares closely to the value previously obtained from the newly formed synapse, 2.3 $\pm$ 0.6 msec (Kullberg et al, Develop. Biol., ibid.). These results indicate that the developmental decrease in MEPC rise time is solely due to increased AChE activity and decreased AChR channel open time. Developmental changes in AChR density, diffusion distance, release kinetics or other factors presumably do not contribute to the change in rise time.

36.10 THE EFFECTS OF DC CURRENT INJECTION ON IN SITU AXONAL GUIDANCE AND SYNAPTOGENESIS. G.Q.Fox, D.Kötting* and G.P.Richardson* Dept. Neurochemistry, Max-Planck-Inst. für biophysikalische Chemie, 3400 Göttingen, FRD.

Embryonic and neonatal Torpedo marmorata were used to study the effects of DC current injection on axonal guidance and synaptogenesis within their electric organs. The electric organ is so structured that current can be directed in a parallel axis to synaptic terminal formation and perpendicular to axonal growth. In vitro studies have shown that neurites will preferentially guide towards the cathode when grown within an electric field. Data are also available that show that growth cones generate ionic currents and that such currents appear to be a prelude to the establishment of a variety of developmental growth patterns. In our system, therefore, current injection would be postulated to effect synaptic terminal formation but not ingrowth of axons, which occurs at right angles to current flow.

Cationic probe studies with ruthenium red, alcian blue/lanthanum and lysozyme demonstrate that the ventral electrocyte surface including the basal lamina has relatively more anionic sites than the dorsal surface. As this surface is the exclusive post-synaptic locale, its cathodal nature is consistent with the hypothesis.

Estimated current densities of 1-100$\mu A/cm^2$ were injected in a ventral-dorsal(top $\ominus$) and dorsal-ventral (top $\oplus$) direction. Samples of electric organ were then evaluated quantitatively at the electron microscopic level for percent neuritic coverage of postsynaptic membrane. Top $\ominus$ currents, predicted to retard percent coverage, did so in an apparent strength-duration manner with a current density of $10\mu A/cm^2$ applied for 2 weeks being the minimum necessary to evoke a statistically significant response. Top $\oplus$ injections, predicted to enhance percent coverage, appear to do so only under maximal tolerable current densities for extended periods of time(e.g. $50\mu A/cm^2$ for 4 weeks). No evidence was obtained to suggest that the ingrowth of axons was in anyway effected by the currents in either direction.

In conclusion, the results support a hypothesis of synaptogenesis which must take into consideration galvanotropic effects.

36.11 APPEARANCE AND DEVELOPMENT OF CHEMOSENSITIVITY OF EMBRYONIC AMPHIBIAN SPINAL NEURONS IN VITRO. Nicholas C. Spitzer & John L. Bixby, Biology Dept., UCSD, La Jolla, CA 92093

The development of excitable membrane properties has been investigated in the Rohon-Beard neurons of the Xenopus spinal cord. Cultures prepared from neural plate stage embryos contain a population of neurons that is likely to include Rohon-Beard cells and motoneurons, on the basis of their early birthdate and profiles of sensitivity to neurotransmitters. The differentiation of the action potential of these neurons parallels the development of the impulse in Rohon-Beard neurons in vivo. We have examined the development of chemosensitivity of these cells isolated in culture. Low density cultures are prepared by dissociating the spinal region of the neural plate in Ca^{++}/Mg^{++}-free saline, and cells are grown in a defined medium without exogenous organic components. Neurons can be recognized by their morphology as early as six hr. in vitro, and impaled with intracellular microelectrodes.

Neurons are initially insensitive to bath application of GABA, but are depolarized in association with a conductance increase as early as 8½ hr in culture, corresponding to Nieuwkoop & Faber stage 24 for intact siblings from the same clutch of eggs. This time of onset is indistinguishable from that reported for the Rohon-Beard neurons in vivo (Bixby & Spitzer, J. Physiol., in press). As in vivo, a fraction of these cells are also depolarized by glycine. Furthermore, cells that are hyperpolarized by GABA and glycine become sensitive at about the same time. This is the chemosensitivity profile described for adult frog motoneurons.

The sensitivity of the cells was examined by iontophoretic application of GABA to the soma during the first 24 hr in culture; sensitivities as high as 180 mV/nC have been observed, which are comparable to those observed for Rohon-Beard neurons in vivo at this stage of development. The reversal potentials of these iontophoretic responses were determined by injecting current to shift the membrane potential to different levels; voltage dependent conductances were suppressed pharmacologically. Neurons have reversal potentials of either -35 or -60 mV, which are similar to the values reported for Rohon-Beard neurons and motoneurons in vivo, respectively. The latter class of neuron can functionally innervate skeletal muscle cells in these cultures. The depolarizing response to GABA is reduced or blocked by 10 µM picrotoxin or curare, like that of Rohon-Beard neurons in the spinal cord, as is the hyperpolarizing response of the other cells. Thus the development of sensitivity to neurotransmitters of some of these neurons in dissociated cell culture parallels the development of Rohon-Beard neurons in vivo, with respect to the time of onset, degree of sensitivity, reversal potential and pharmacology of the responses to GABA. In addition, putative motoneurons developing in these cultures become sensitive to neurotransmitters at roughly the same stage as the Rohon-Beard neurons.

36.12 DEVELOPMENT OF A HIGH AFFINITY GABA UPTAKE SYSTEM IN EMBRYONIC AMPHIBIAN SPINAL NEURONS IN CULTURE. Janet E. Lamborghini and Amanda Iles.* Biology Dept. B-022, UCSD, La Jolla, CA 92093

The differentiation of several neuronal membrane properties has been studied in Rohon-Beard neurons developing in vivo. The onset and development of electrical excitability and chemosensitivity have been described for these amphibian spinal neurons, as well as the electrical uncoupling of these cells. The development of a heterogenous population of spinal neurons which include the Rohon-Beard neurons has been examined in dissociated cell culture. The development of the action potential under these conditions parallels its development in vivo. These cultures thus appear to be a useful model system for the study of the mechanisms underlying neuronal differentiation. A high affinity uptake system for neurotransmitter precursors is another characteristic neuronal membrane property. Here we describe the evaluation of a high affinity uptake system for GABA and its development in neurons in vitro.

Cultures were prepared from Xenopus neurulae. Uptake was assayed by exposing the cells to (^{3}H)-GABA for one hour and processing the cultures for autoradiography. Uptake by 24 hour old cultures occurred by a high affinity mechanism because 1) 35% of neurons were labeled by exposure to 10^{-7} M (^{3}H)-GABA, 2) the percentage of neurons labeled was greatly reduced by treatment with 10^{-5}M ouabain, 3) labeling was blocked by replacement of the Na^+ in the medium with Li^+, and 4) labeling was blocked by incubation at 0°C.

The development of this high affinity uptake system for GABA was studied by assaying its presence at different times after the cultures were prepared. Fewer than 6% of neurons showed labeling at 6 and 12 hrs in culture. The percentage of cells accumulating GABA rose steeply between 12 and 18 hrs to 35% and remained at that value through 24 hrs. This suggests that the ability to accumulate GABA by a high affinity uptake system develops in this population of cultured neurons between 12 and 18 hours in culture. This period is several hours later than that in which cultured neurons are becoming sensitive to GABA (see Spitzer & Bixby, this volume).

Experiments in vivo demonstrate that Rohon-Beard neurons have the ability to take up GABA, while primary motor neurons do not. Results of previous studies (Spitzer & Lamborghini, 1976) indicate that the majority of neurons in these cultures is composed of Rohon-Beard neurons and primary motor neurons. These facts together suggest that at least some of the neurons which accumulate GABA in our cultures are Rohon-Beard neurons.

The presence of a high affinity GABA uptake system in neurons developing in culture raises the possibility that the neurons use this compound as a neurotransmitter.

Supported by grants from the NIH and the ONR.

37.1 SELECTIVE STAINING OF CELLS IN TREE SHREW AND GRAY SQUIRREL RETINAE FOLLOWING INTRAVITREAL INJECTION OF PROCION YELLOW DYE. Heywood M. Petry, Dept. of Psychology, Vanderbilt University, Nashville, TN, 37240

The tree shrew (Tupia glis) and eastern gray squirrel (Sciurus carolinensis) are diurnal mammals which occupy similar ecological niches. Both have cone dominated retinae and exhibit dichromatic color vision. The visual system of these species differs, however, in at least three important respects. First, tree shrew color vision resembles that of human deuteranopes, while that of the gray squirrel is similar to human protanopes. Second, rods account for less than 4% of the receptors in the tree shrew retina, whereas 35-40% of squirrel receptors are rods. Third, while receptors are arranged in a single retinal layer in the tree shrew, gray squirrel receptors are organized into two tiers: rods being located in the inner, more vitreal tier, and cones being located in the outer tier (West & Dowling, J. comp. Neurol., 1975, 159, 439). The overall similarities in the size and lifestyle of these animals, coupled with the differences in their retinal organization and color vision prompted this study of a feature common to the visual systems of both species: the short-wavelength sensitive cone system.

To anatomically identify the short-wavelength sensitive cones, 10-15 μl of Procion Yellow M4RF (7% in deionized water) was injected into the vitreous humor of anesthesized tree shrews and gray squirrels. A similar procedure has been shown to selectively and completely stain short-wavelength sensitive cones in the monkey retina (de Monasterio et al., Science, 1981, 213, 1278). After a 20-25 hour period, the tree shrews and squirrels were perfused with formalin, and retinal pieces were dehydrated, defatted, and embedded in EPON. Sections were cut at a thickness of 10 microns in either the radial or tangential plane and observed under episcopic fluorescence. In both species, cone outer segments were stained routinely. In addition, a small, regularly spaced population of receptors, as well as horizontal, bipolar, and ganglion cells, were completely stained by the dye. Completely stained cells were more common in central retina, although they regularly occurred in peripheral regions as well. In the tree shrew all stained receptors appeared to be cones. However, in the squirrel retina, completely stained receptors were located in the inner "rod" tier. This presents two intriguing possibilities. Either Procion Yellow is taken up by rods in this species, or there is a population of presumably short-wavelength sensitive cones located in the squirrel's "rod" tier. Regardless, the results suggest that although both species are diurnal dichromats occupying a similar niche, the organization of their cone and rod systems is very different. (Supported by EY-04239 to H.M. Petry and EY-01778 to V.A. Casagrande)

37.2 EXCITATORY AMINO ACIDS SUBSERVE PHOTORECEPTOR AND ON BIPOLAR NEUROTRANSMISSION. M. M. Slaughter* and R. F. Miller (SPON: A. I. Cohen). Dept. of Ophthal., Physiol. and Biophys., Washington Univ. Sch. of Med., St. Louis, Mo. 63110.

The effects of ± cis 2,3 piperidine dicarboxylic acid (PDA), a putative excitatory amino acid antagonist, were examined in the superfused retina-eyecup preparation of the mudpuppy using intracellular recording techniques. We found that PDA was a general excitatory amino acid antagonist that opposed the depolarizations evoked by aspartate, glutamate, kainic acid, and N methyl aspartate. In horizontal cells, PDA blocked the kainic acid induced depolarization but not the GABA induced depolarization, indicating a selective action of PDA. In the outer retina, PDA essentially blocked the light response of horizontal cells and OFF bipolars. This was associated with a hyperpolarization similar to that caused by the use of cobalt to block photoreceptor transmitter release. The center response of the ON bipolar was unaffected by PDA while the antagonistic surround was blocked, as would be expected from PDA's action on horizontal cells. In the inner retina, both ON and OFF light responses were diminished by PDA. The block of the OFF responses could be explained by PDA's effect on the OFF bipolar. But, since the ON bipolar functions normally, PDA must block the ON bipolar neurotransmitter. Conductance measurements indicate that the block of the light responses was not due to a shunting action by PDA.

Several selective aspartate antagonists (D α amino adipate, D α amino subrate, and D γ glutamyl-glycine) were used in an attempt to identify synaptic pathways that utilize this excitatory amino acid neurotransmitter. These agents did not block photoreceptor or bipolar cell transmission. We conclude that photoreceptors release an excitatory amino acid, probably glutamate, that interacts with two general types of post-synaptic receptors: one on the ON bipolar and the other on the horizontal cell and the OFF bipolar. In addition, the ON bipolar releases an excitatory amino acid neurotransmitter. The OFF bipolar may utilize the same neurotransmitter as the ON bipolar, but this cannot be evaluated with this protocol.

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37.3 LIGHT AND DARK-DEPENDENT RELEASE OF GLUTAMATE AND ASPARTATE IN THE ISOLATED RETINA OF THE MUDPUPPY. R.F. Miller, M.M. Slaughter* and S.C. Massey* Departments of Ophthalmology, Physiology and Biophysics, Washington University School of Medicine, St. Louis Mo 63110.

We have analyzed the amino acid content of perfusate fluid collected from the isolated retina of the mudpuppy. Reverse-phase high pressure liquid chromatography was used for separation and identification of amino acids using an O-phthalaldehyde derivitization procedure and flurometric detection. An isolated mudpuppy retina was moved between 100uL wells containing a Ringer's solution combined with various pharmacological agents. The use of DL-2-amino-4-phosphonobutyrate(APB) and cis 2,3 piperidine dicarboxylate (PDA) provides a preparation in which light modulated neuronal activity is restricted to the photoreceptors. When the retina is bathed in APB and PDA containing Ringer's, glutamate and aspartate release are readily detected. Continuous dark release of glutamate and aspartate is significantly reduced in cobalt or low Ca++, high Mg++ Ringer. A strong bleaching light causes modest changes in glutamate release:A light evoked increase and decrease have been seen. A striking increase in glutamate release is observed during dark adaptation following the bleaching light. The enhanced glutamate release is blocked by low Ca++, high Mg++(20mM). Aspartate release is comparatively unaffected by the light vs dark cycle. These observations are discussed in view of neuropharmacological evidence from this laboratory which favors glutamate as a photoreceptor transmitter.

37.4 THE ACTIONS OF ASPARTATE, GLUTAMATE, AND THEIR ANALOGS ON RETINAL NEURONS OF THE RABBIT, Stewart A. Bloomfield* and John E. Dowling. The Biological Laboratories, Harvard University, Cambridge, MA 02138.

Previous investigations in lower vertebrate retinas have suggested that the acidic amino acids aspartate (L-Asp) and glutamate (L-Glut) are neurotransmitters released by the photoreceptors. We have initiated a study of the actions of these agents and their analogs on the retinal neurons of a mammal. The effects of these drugs were evaluated according to their modulation of the intracellularly-recorded responses of neurons in the superfused, isolated retina-eyecup of the rabbit (Dacheux et al., Fed. Proc.:32, 1973).

Horizontal cells (HCs) displayed equal sensitivity to L-Asp and L-Glut. At concentrations of 2.5 mM and greater, these agents caused a depolarization of the dark membrane potential and an attenuation of the light-evoked responses; actions consistent with that expected of the endogenous photoreceptor transmitter. Kainic acid (KA), a structural analog of glutamate, had a similar effect on HCs but at concentrations as low as 50 μM. HCs were less sensitive to N-methyl-DL-aspartate (NMDLA), a structural analog of aspartate; depolarizing effects could be elicited only with applied concentrations of 100 μM or more. When exposed to low concentrations of NMDLA (50 μM), about 65% of the HCs hyperpolarized. This effect could not be reversed with the simultaneous addition of 50 μM KA to the perfusate, suggesting that low concentrations of NMDLA antagonized the effect of KA.

Preliminary results show that ON-bipolar cells are hyperpolarized by 50 μM KA and are insensitive to 50 μM NMDLA. OFF-bipolars are strongly depolarized by 50 μM KA and are hyperpolarized by 50 μM NMDLA. As was the case for HCs, this hyperpolarizing action of low concentrations of NMDLA could not be reversed by the simultaneous application of KA.

In the inner retina, amacrine and ganglion cells were considerably more sensitive to KA than NMDLA. Following the application of 50 μM KA or 100 μM NMDLA many cells displayed an initial increased spontaneous spike activity followed by a large depolarization of the dark membrane potential which abolished both spiking and the light-evoked responses.

37.5 EFFECTS OF AMINO ACID DRUGS ON RESPONSE PROPERTIES OF FISH HORIZONTAL CELLS. S. C. Mangel*, M. Ariel* and J. E. Dowling. (SPON: E. M. Lasater). The Biological Laboratories, Harvard University, Cambridge, MA 02138.

Response properties of fish horizontal cells were investigated before and after the application of glutamate and aspartate agonists and antagonists. Drugs were applied to the isolated carp retina while recording intracellularly from horizontal cells. As reported previously, glutamate and aspartate depolarize the membrane potential of all types of dark-adapted horizontal cells, an action which is consistent with that of the endogenous photoreceptor transmitter. However, N-methyl-DL-aspartate (NMDLA), a structural analogue of aspartate that acts as an aspartate agonist on spinal cord motorneurons (Watkins et al., 1981), hyperpolarizes the membrane potential of dark-adapted horizontal cells and decreases the amplitude of their light-evoked responses. These effects of NMDLA are not expected of a photoreceptor transmitter agonist, but rather suggest that NMDLA is an antagonist of the photoreceptor transmitter(s).

The effects of NMDLA are indistinguishable from those of alpha methyl glutamic acid, a glutamate antagonist, and alpha amino adipic acid, an aspartate antagonist. Furthermore, during the hyperpolarization of L-type cone horizontal cells produced by NMDLA and the two antagonists, an increase in the response to diffuse blue light occurs, as well as a decrease in the response to diffuse red light. Because application of Co^{++} produces a hyperpolarization with no concomitant spectral difference, the blocking action of these antagonists may reveal that red cones release a different transmitter than blue cones. The blue response increase that occurs following application of acidic amino acid antagonists does not occur when small spot stimuli are used. Therefore, these antagonists may reveal the presence of a surround feedback from L-type (H1) horizontal cells which is wavelength dependent. Possible synaptic pathways will be discussed.

37.6 DOPAMINE (DA) AND DRUGS THAT INCREASE INTRACELLULAR CYCLIC AMP DECREASE JUNCTIONAL COMMUNICATION BETWEEN L-HORIZONTAL CELLS. J. Neyton*, M. Piccolino* and H. M. Gerschenfeld* (SPON: ENA) Lab. Neurobiol., Ecole Normale Supérieure, 75005 Paris, France and Istituto Neurofisiologia, C.N.R., 56100 Pisa, Italy.

The axon terminals of L-horizontal cells of the Pseudemys turtle retina, which correspond to the large field horizontal cell (L1HC) of Simon, are coupled by gap-junctions and thus form an extensive network. We have reported (Piccolino et al.,PNAS, June '82) that micromolar concentrations of bicuculline and picrotoxin evoke a narrowing of the receptive field profile of the L1HC. This effect is accompanied by a marked decrease in the diffusion of the Lucifer yellow dye in the L1HC network and is rapidly reversed by GABA. When GABA was applied alone at 0.5-1 mM it increased the peripheral contribution to the L1HC responses. We interpreted the effects of the GABA antagonists as resulting from a decrease in the conductance of the gap-junctions of the L1HC network.

DA (0.2-10 µM) as well as its agonists epinine (1-10 µM), apomorphine (20-40 µM) and ADTN (1 µM) also evoke a narrowing of the receptive field profile of the L1HC. The DA-antagonists cis-Z-flupenthixol (50 µM) and piflutixol (50 µM) when applied previous to the amine treatment, prevent the effects of DA (10 µM).

Since DA was shown to increase cyclic AMP concentration of isolated horizontal cells of fish retina (van Buskirk and Dowling PNAS 78,825,1981), we investigated the effects of other drugs that increase intracellular cyclic AMP concentration. Forskolin (5 µM), a stimulator of adenylate cyclase (Seamon et al.,PNAS 78, 3367,1981) and isobutylmethylxanthine (IBMX, 20-50 µM), an inhibitor of phosphodiesterases, both also evoke the shrinking of the L1HC receptive field profile. However, IBMX may also cause an increase of sensitivity and a slowing down of the L1HC responses. Moreover, both DA and forskolin induce a remarkable restriction of the diffusion of Lucifer yellow in the L1HC network.

Our experiments suggest that both the receptive field narrowing and the restriction of Lucifer yellow diffusion evoked by DA and its agonists are probably due to a decrease in the conductance of the gap-junctions in the L1HC network in which cyclic AMP may be involved as a second messenger. A possible antagonistic interplay between a GABAergic and a dopaminergic system intervening in the regulation of the permeability of the gap-junctions between L1HC and thus in their receptive field properties is being investigated.

37.7 EFFECTS OF 6-HYDROXYDOPAMINE ON THE SPATIAL PROPERTIES OF CARP HORIZONTAL CELLS. J.L. Cohen and J.E. Dowling. Dept. of Ophthal., New York Univ. Med. Ctr., New York, NY 10016 and Bio. Labs, Harvard Univ., Cambridge, MA 02138.

The perikarya of the interplexiform cell lies in the inner nuclear layer amongst the amacrine cells. In teleost fishes it makes connections presynaptically to horizontal and bipolar cells in the outer plexiform layer, while in the inner plexiform layer it makes both pre- and postsynaptic contacts with amacrine cells. The interplexiform cell has been shown to contain the neurotransmitter dopamine. Very little information exists as to its physiological role. Recent evidence (Negishi & Drujan, 1979) suggests that dopamine may influence the spatial properties of the horizontal cells. We therefore wanted to determine what effect the removal of dopamine had on the spatial properties of the horizontal cells as well as to elucidate any possible mechanisms of action.

Carp were injected in one eye with 10 µl of 1 mg/ml 6-OHDA in 0.9% NaCl with 1 mg/ml ascorbate added as an antioxidant. Injections were made on two consecutive days. Intracellular recordings from the horizontal cells were made on day seven. Receptive field diameters were determined by recording the response of the horizontal cells to spots of light of increasing diameter. Control retinas responded linearly to spots of increasing size up to about 2.5 mm in diameter. Stimulation with larger diameter spots had no effect on response amplitude. Retinas treated with 6-OHDA responded linearly throughout the range of stimuli sizes used. We therefore conclude that depletion of dopamine in the carp retina increases the diameter over which a test stimulus will elicit a response from the horizontal cell.

To try to determine the mechanism of action of dopamine on the spatial properties of the carp horizontal cells we tested the effects of dopamine and 8-bromoadenosine 3'5' monophosphate (8-bromocAMP) on the horizontal cells. Horizontal cell responses to spots and annuli were made equal with neutral density filters. Dopamine (10-100 mM) and 8-bromocAMP (0.25-25 mM) were sprayed on the retina by means of an atomizer. Responses to spot stimuli increased in amplitude, while surround responses decreased in some cells. The change in receptive field size in these cells was not accompanied by a consistent membrane potential change. These preliminary results therefore suggest that dopamine exerts its effects on receptive field size indirectly, i.e., via cAMP.

37.8 DEVELOPMENT AND CONNECTIVITY OF PUTATIVE CHOLINERGIC AMACRINE CELLS IN RABBIT RETINA. Edward V. Famiglietti, Jr. Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201

Litters of pigmented rabbits were born in a conventional animal care facility, and time of birth was established to within 8-16 hrs. Animals were sacrificed at timed intervals; their retinas were flat-mounted and taken through a Golgi-Kopsch impregnation procedure. At 5 days of age, starburst (Sb) amacrine cells, the putative cholinergic amacrine cells of mammalian retina, were recognizable by the radial symmetry and regular branching of their stellate dendritic trees. They were very different from adult Sb amacrines, however, in having sinuous dendrites of conventional (gradual) taper, virtually no proximal-distal differentiation in dendritic contour, and numerous irregular spinous processes 1-8 um in length along the course of the dendrites. At this stage, ganglion cells exhibit a similar degree of immaturity, and cannot be readily classified by criteria developed for adults. Bipolar cells have dendritic terminals in the outer plexiform layer and bushy axon terminals in the inner plexiform layer (IPL), even though synaptic ribbons have not been seen in the IPL at this stage (McArdle, C.B., J.E. Dowling, and R.H. Masland, J. Comp. Neur., 175:253, 1977).

At 8 days of age, Sb amacrines have assumed features of adult cells; in particular, a beginning of the tripartite annular organization of the dendritic tree is evident, i.e. a proximal zone of conventionally tapering dendrites, an intermediate zone of slender, smooth dendrites, and a distal zone of beaded dendrites with boutons en passant and boutons terminaux (Famiglietti, E.V., Invest. Ophthalmol. Suppl., 20:204, 1981).

At 10 days, Sb amacrines are virtually indistinguishable from adult cells, and adult types of ganglion cells are easily recognized by their branching patterns. Nevertheless, the ganglion cells retain immature dendritic spinous appendages seen at earlier stages. Despite the presence of immature spines and contours of their axon terminals, rod bipolars and more than one type of cone bipolar can be distinguished at this stage.

Golgi-impregnated Sb amacrine cells have been examined by electron microscopy in adult rabbits; they receive bipolar input in the proximal and intermediate dendritic zones, while their distal zone of boutons delivers extensive output to ganglion cell dendrites. These distal dendrites are involved to a small degree in amacrine-to-amacrine interactions as well. This simple polarization of synaptic connectivity in Sb amacrines, odd though it seems in adult cells with their very slender (0.2 to 0.3 um) intermediate dendritic segments, may be established at an early developmental stage in which the dendritic tree has a more conventional appearance, resembling that of ganglion cells.

37.9 PSEUDOCHOLINESTERASE AND ACETYLCHOLINESTERASE STAINING IN CAT AND MONKEY RETINA. R.-B.Illing* and A.M. Graybiel, Dept. of Psychology and Brain Science, Mass. Inst. Technology, Cambridge, MA 02139.

Twenty-two cat and 7 monkey retinae were reacted for either pseudocholinesterase (BuChE, using butyrylthiocholine iodide as substrate) or acetylcholinesterase (AChE) in the presence, respectively, of the inhibitor of the other enzyme.

In the cat retina, AChE activity was found in all categories of cells in the ganglion cell layer (GCL), including all types of ganglion cells, displaced amacrine cells and microglia. In whole mounts, the radial pattern formed by ganglion cell fibers could be seen up to the optic nerve head. In cross-sections, 3 sublaminae of the inner plexiform layer (IPL) could be distinguished, the middle band being pale. No AChE activity appeared in other layers. BuChE histochemistry allowed us to distinguish distinct types of retinal cells. Three of these appear to be amacrine cells(B1-B3). B1 cells had soma diameters of 13-15μm, were situated at the inner border of the inner nuclear layer (INL), and had dendritic trees that were sparsely branched in the outer IPL and stained for up to a diameter of 400μm. B2 cell bodies lay in the same plane of focus as the B1 cells, but had smaller diameters (11μm) and richly branched dendritic trees (>200μm dia). There were hints that other cell types in the INL might also be BuChE-positive. B3 cell bodies lay in the GCL but they appeared to be amacrine cells because they were not peroxidase-positive after massive HRP injections into the lateral geniculate, superior colliculus and pretectum. They had spherical 10-12μm somas, no nuclear fold and branched dendritic arbors of 300μm. As with AChE, BuChE staining clearly showed the ganglion cell fiber pattern in whole mounts. Unlike AChE preparations, BuChE stained only a few ganglion cells strongly; these had β and γ cell profiles. In sections, the IPL was diffusely stained, most heavily near the INL. The outer nuclear layer (ONL), receptor inner segments and outer limiting membrane were also stained. Müller cells were BuChE-positive and their inner endfeet formed a gridwork in the axon layer.

In monkey, AChE stained all cells in the GCL, as in cat; split the IPL into 5 bands; and stained the inner part of the outer plexiform layer (OPL). BuChE stained at least B1 cells and B3 cells. Of cells in the GCL, those with the largest profiles were apparently not stained. IPL staining was split into 5 bands, OPL staining into 3 bands.

We conclude that BuChE as well as AChE is a differential marker for retinal sublayers and that BuChE activity in particular marks at least 3 populations of amacrine cells and probably ganglion cell types in the small and medium size range as well.

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37.10 MORPHOLOGICAL, PHARMACOLOGICAL, AND PHYSIOLOGICAL CHARACTERIZATION OF AMACRINE CELLS IN THE MUDPUPPY RETINA. P. A. Coleman* and T. E. Frumkes. Dept. Psychology, Queens College of CUNY, Flushing, New York 11367.

Current models of the vertebrate retina maintain that ganglion cells receive an excitatory input from the depolarizing bipolar cells at light on and from the hyperpolarizing bipolar cell at light off. In addition, an inhibitory on-off input from amacrine cells has been shown to modify ganglion cell discharge. In the mudpuppy, this characterization has been made on the basis of pharmacological and physiological evidence alone.

In the present study, intracellular responses were obtained from neurons of the superfused mudpuppy retina with electrodes fashioned out of triangular glass and filled with Horseradish Peroxidase. These electrodes provide a combination of technical advantages including easy filling, relatively low impedance (facilitating extrinsic current injection and yielding superior physiological recordings), and the ability to characterize cells anatomically. The physiological and pharmacological characteristics of on-off amacrine cells prove similar to those described earlier: accordingly, these neurons rarely spike to maintained illumination, have high sensitivity to exogenously applied GABA and glycine but show no signs of on-off inhibition, and have large receptive field with no obvious signs of center-surround antagonism. The dendrites of these neurons arborize diffusely throughout the inner plexiform layer (IPL) and cover an extremely large expanse ($\gg$500 mμ). On amacrine cells also rarely spike to maintained illumination and are sensitive to exogenously applied GABA and glycine but in other respects differ from on-off amacrines. Specifically, On amacrines have small ($<$300 mμ) receptive fields and depolarizing current injection reveals transient inhibition at the onset and offset of photic stimulation. The dendritic field of these neurons (80-200 m diameter) is restricted to a narrow ($<$5 mμ) plane of the IPL very close to the ganglion cell bodies. Our results suggest that On amacrines are inhibited by GABA and/or glycine releasing on-off amacrines and in turn provide On inhibitory input to postsynaptic neurons. Results are compared with data from other types of identified amacrine cells in mudpuppy and with the synaptic structure of the IPL in other species.

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37.11 COMPARISON OF EFFECTS OF KAINIC ACID ON SOMATOSTATIN, SUBSTANCE P AND DOPAMINE IN THE RABBIT RETINA. S.M. Sagar, L. Weinstein* and J.B. Martin. Neurology Service, Massachusetts General Hospital, Boston, MA 02114.

We have previously documented the presence of somatostatin-like immunoreactive material (SLI) in the rabbit retina. In this study we report an unexpected failure of the neurotoxin kainic acid (KA) to deplete the rabbit retina of more than 50% of its content of SLI. Groups of adult, male Dutch belted rabbits were sedated with pentobarbital, 20 mg/kg IV, and received injections of varying doses of KA (30-240 nmol) intravitreally into one eye and saline vehicle into the other. One week later the rabbits were killed and their retinas removed. A segment of each retina was removed for histological examination and the remainder was extracted and assayed for SLI and substance P-like immunoreactivity (SP) by radioimmunoassay, dopamine (DA) by HPLC with electrochemical detection and protein. Histologically, KA produced destruction of the inner layers of the retina in a dose-related manner, with preservation of the photoreceptors, and with a dose-related depletion of retinal protein content. At KA doses of 60-240 nmol, both SP and DA content were reduced to 3-21% of control values. The dose-response curve for SLI depletion was approximately parallel to those of SP and DA, but the greatest mean depletion of SLI observed was 50%. This failure to reduce retinal SLI content by more than 50% at doses of KA which produce severe histologic damage to the neural retina and dramatic depletions of SP and DA content demonstrates that the SLI-containing structures of the rabbit retina, presumably a subclass of amacrine cells, are partially resistent to the neurotoxic action of KA. This result is in marked contrast to results reported in rat and chick. We hypothesize that SLI in the rabbit retina is contained in two pharmacologic types of cells in about equal abundance; one type is sensitive to and one type insenstive to KA. This hypothesis, if verified, would have importance in investigating the mechanism of action of KA and the pharmacology of somatostatin-containing neurons of the retina.

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37.12 MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED RABBIT RETINAL GANGLION CELLS. F. R. Amthor, C. W. Oyster, and E. S. Takahashi. Dept. of Physiological Optics, University of Alabama in Birmingham, Birmingham, Alabama 35294.

Intracellular recordings and iontophoretic HRP injections were made in rabbit retinal ganglion cells in a superfused eyecup preparation. The morphologies of these cells were analyzed in flat mount preparations. Inner plexiform layer (IPL) ramification of the cell's dendrites were determined with the aid of three dimensional computer reconstruction.

We report a variety of cells with concentrically organized receptive fields as well as several more complex types. ON and OFF center concentric cells were found with at least two and possibly three distinct morphologies corresponding to previously identified anatomical classes. At least one anatomical class (radial dendritic morphology), which has subgroups whose dendrites branch in either sublamina a or b, can now be associated with a physiological class (concentric cells) which are either OFF center (dendrites in sublamina a) or ON center (dendrites in sublamina b), respectively. All concentric cells found so far appear to obey this a/b (OFF/ON) sublamination scheme.

An ON-OFF cell having attributes of the local-edge-detector physiological class has been found to have a dense and intricate branching pattern which is globally bistratified and possibly tristratified. The morphology of this cell may be distinct from morphological types we have previously reported, but is similar to an ON-OFF cell reported by Bloomfield & Miller, (1981).

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38.1 IN VITRO LABELING RECEPTOR AUTORADIOGRAPHY: LOSS OF LIGAND DURING PREPARATION. M.J. Kuhar and J.R. Unnerstall. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Balto., MD 21205

In vitro labeling autoradiography involves 1) incubating slide mounted tissue sections with ligands (LIG) to label receptors with a high degree of specificity, and 2) generating autoradiograms by a procedure which does not allow a significant loss of, or diffusion of, ligand from receptor. Young and Kuhar (Brain Res. 179:255, 1979) dealt with the second point by avoiding contact with aqueous or organic media and by directly apposing labeled, slide-mounted tissue sections (LASEC) to dry, emulsion-coated coverslips. Subsequently, others suggested modifications of this which involved fixation of the LASECs with hot aldehyde vapors, dehydration and defatting of the LASECs in ethanols and xylene, and direct dipping of the LASECs into hot, molten emulsion. It was implied that at least some LIGs were fixed to the receptors by the aldehyde treatment. In this study, we have tested for the loss of several reversible receptor labeling LIGs from formaldehyde vapor fixed LASECs in ethanol solutions.

LASECs were made by routine procedures. They were fixed in a heated, closed vessel containing paraformaldehyde. After various treatments, the tissue sections were scraped from the slides for determination of specific binding.

Fixed LASECs were soaked for various times in 70% ethanol. Every LIG examined was lost from the section in significant quantities. ^{3}H-Naloxone and ^{3}H-muscimol showed the least loss which was 30-40%. ^{3}H-Dihydromorphine, ^{3}H-para-aminoclonidine, ^{3}H-prazosin and ^{3}H-spiperone showed 70-80% loss. ^{3}H-n-Methylscopolamine (NMS) was totally lost. Changes in fixation conditions could reduce but not prevent the loss of some LIGs. The bulk of the loss occurred by one minute after which it was more variable and slower.

Several experiments were carried out with NMS in attempt to prevent total loss of ligand from LASECs. Other reagents were used as fixatives, and LASECs labeled with NMS were dipped directly into molten emulsion (Kodak NTB-3) without any exposure to ethanol or xylene; there was still a total loss of label within 60 seconds.

Thus, the introduction of dehydration, defatting and wet emulsion application into in vitro labeling procedures is not without problems, even in fixed tissues. The loss of ligand, which varied widely depending on the ligand used, indicated the possibility that diffusion of ligand will occur in the section to the extent that anatomical resolution will be reduced. Even if this latter were not a possibility, longer exposure times will be necessary. The total loss of at least one LIG indicates that these procedures are not generally applicable.

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38.2 LOCALIZATION OF SEROTONIN-LIKE IMMUNOREACTIVITY IN THE LOBSTER HOMARUS AMERICANUS. B.S. Beltz and E.A. Kravitz. Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115.

The amine serotonin (5HT) is found in lobsters both in central ganglia and in association with the neurosecretory neurons and pericardial organs (PCOs) of a diffuse peripheral neurohormonal network. This peripheral network, which is found along the second nerve roots of thoracic and subesophageal ganglia, releases at least three physiologically important compounds (5HT, octopamine, and the peptide proctolin) into the haemolymph. Among its many peripheral actions, circulating 5HT induces a contracture and enhances the strength of nerve-evoked contractions in lobster exoskeletal muscles. In addition, we believe that 5HT released from central synaptic sites can cause a rigid flexion of the legs and abdomen, similar to the posture seen when 5HT is injected at high concentrations into freely moving lobsters (see Kravitz et al. (1981) in Serotonin Neurotransmission and Behavior, eds. Jacobs and Gelperin, pp. 190-210).

To explore the peripheral and central actions of 5HT at a cellular level, we require a detailed knowledge of the locations of serotonergic cell bodies and release sites. To this end, we have used immunohistochemical techniques in wholemount preparations to explore the distribution of 5HT-like immunoreactivity in the lobster nervous system. The specificity of our antibody (obtained from Immunonuclear Corp.) was demonstrated by showing that immunoreactivity was lost: (1) when antibody solution was pre-absorbed with a 5HT-albumin conjugate (the original antigen) or with 5HT and (2) when 5HT was depleted from tissues by treatment with the neurotoxin 5,7-dihydroxytryptamine.

With this method we found over 100 cell bodies that contain 5HT-like immunoreactivity. A few of these are located peripherally near PCOs, but the vast majority are located in central ganglia. Every ganglion tested contains at least one immunoreactive cell body. Approximate cell body counts per ganglion are: 34-brain; 1 per circumesophageal ganglion; 26-subesophageal ganglion; 2 per thoracic ganglion; 3-5 per abdominal ganglion. Pairs of cell bodies in the 5th thoracic and 1st abdominal ganglia send processes out the thoracic second roots to form part of a dense plexus of fine fibers and varicosities that surrounds the roots and PCOs. The neurosecretory neurons located at the bifurcations of the second roots do not show any immunoreactive staining.

With the wholemount preparations, we have been able to trace the projections of many of the immunoreactive neurons, and have constructed a map of this system of cell bodies, fibers and endings in Homarus. We are using this map to locate the same cells in living preparations in order to examine their relationships to the peripheral neurohormonal network and the generation of particular postures. (Supported by NIH).

38.3 IMMUNO-REACTIVE MYELINATED 5-HT AXONS IN THE MEDIAL FOREBRAIN BUNDLE OF MONKEY AND RAT. Efrain C. Azmitia and Patrick J. Gannon, Dept. of Anatomy, Mt. Sinai Sch. Med. N.Y., N.Y. 10029

Ascending monoaminergic fibers, classically described as unmyelinated (Dahlstrom and Fuxe ,Acta physiol. scand. 62, 1, 1964; Beaudet and Descarries, J. physiol. Paris, 77, 193, 1981) use the MFB as their major route. We here report the presence of myelinated axons immuno-reactive to an antibody raised against 5HT-hemocyanin (gift of J. Lauder).

Monkeys and rats were pretreated with pargyline (50 and 200 mg/Kg, respectively) and L-tryptophan (50 and 200 mg/Kg respectively) before intracardial perfusion with 4% paraformaldehyde, 0.2% glutataldehyde and 0.1% $MgSO_4$ in 0.1M phosphate buffer (20°C). After post fixation in the same fix without glutaraldehyde, 20 um vibratome sections were cut through the hypothalamus and processed by the immuno-peroxidase method. All antibodies were prepared in 0.1M Tris buffered saline) containing 1% normal sheep serum and 0.1% Triton X-100. Sections were pre-incubated in 0.05% 3,3-diaminobenzidine containing 0.2% nickel ammonium sulfate for 10 min at 20°C before the addition of 0.003% hydrogen peroxide. The sections were subsequently post-fixed for 1 hr at 20°C in 2% osmium tetroxide in 0.1M phosphate buffer (pH 7.2) and then block stained in 0.5% uranyl acetate at 5°C for 30 min. No further heavy metal staining was required.

In the monkey 25% and in the rat 1% of the total number of immuno-reactive fibers were myelinated. These fibers ranged between 1.0 - 2.1 um in diameter. Specific labeling was seen in small clear vesicles, the outer mitochondrial membrane , and in patches along the inner axonal membrane. Descending myelinated axons labeled by exogenous ^{3}H-5-HT have been previously reported (Chan-Palay, J. Neurocytol. 7, 419, 1978; Ruda and Gobel, Brain Res. 184, 57, 1980).

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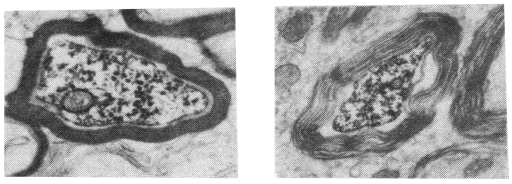

Monkey Rat

38.4 CO-LOCALIZATION OF CHOLINE ACETYLTRANSFERASE AND ACETYLCHOLINESTERASE IN MAMMALIAN FOREBRAIN. A.I. Levey[1], E.J. Mufson, M-M. Mesulam, and B.H. Wainer.[1] University of Chicago,[1] Chicago, IL 60637, and Harvard University, Boston, MA 02215.

Acetylcholinesterase (AChE) has been employed as a histochemical marker for cholinergic neurons despite questionable specificity. We developed monoclonal antibodies against the more specific marker, choline acetyltransferase (ChAT). We now report a comparison of AChE and ChAT localization in the mammalian forebrain. Sections from animals (5 rats, 1 monkey) pretreated with diisopropylfluorophosphate (DFP) were stained either for ChAT by immunoperoxidase, or AChE with silver nitrate intensification, or co-stained for ChAT and AChE in the same section. ChAT was visualized as a diffuse brown reaction product, and AChE as black granules. The procedures were tested in the oculomotor nucleus in the rat and showed that 97% of the cells stained for both markers. In the adjacent serotonergic dorsal raphe nucleus, all the cells were AChE-positive and ChAT-negative.

Major cholinergic projections to mammalian neocortex are believed to arise from a magnocellular system in the basal forebrain that includes the nucleus of the diagonal band of Broca (NDBB) and the nucleus basalis of Meynert (NBM). Double-stained sections of these areas showed that virtually all ChAT-positive cells were AChE-positive; however, these neurons were admixed with significant numbers of ChAT-negative and AChE-positive cells.

Some investigators believe that intensely AChE-stained cells are likely to be cholinergic. The double-labelled cells in the present study exhibited a wide range of AChE-staining intensity; however, intensely-stained cells always co-localized with ChAT. Since the intensity of the AChE stain might be perturbed in the co-localization procedure, cells counts were performed on adjacent sections stained for either ChAT or AChE alone. In the NDBB, there were 62% more ChAT-positive than AChE-rich cells ($P<0.01$), and in the NBM there were 54% more ChAT-positive cells ($P<0.01$). These results suggest that although AChE-rich cells are probably cholinergic, many cholinergic neurons in the basal forebrain do not stain intensely for AChE following DFP treatment. Strikingly different results were obtained in the neostriatum where there was virtually a 100% correspondence between the large AChE-rich cells and ChAT-positive cells. Similar results were obtained in the one monkey forebrain examined. Thus, the use of specific anti-ChAT antibodies to localize cholinergic neurons is likely to be more reliable than AChE histochemistry.

(Support by UPHS-NS17661,[1] HD-04583,[1] the Whitehall Foundation,[1] the Lederer Foundation;[1] and UPHS-NS-09211 and the Essel Foundation.)

38.5 CHOLINERGIC PROJECTIONS FROM THE MESENCEPHALIC TEGMENTUM TO NEOCORTEX IN RHESUS MONKEY. E.J. Mufson, A. Levey, B. Wainer and M-M. Mesulam. Harvard Sch. of Med., Boston, Ma. and Univ. of Chicago, Chicago, Ill.

The nucleus basalis of Meynert is a major source of cholinergic input to the neocortex of the rhesus monkey. There are other non-thalamic subcortical projections into cortex. For example, many neocortical regions receive direct projections from the brainstem Raphe nuclei and from the nucleus locus ceruleus. These nuclei are sources of serotonergic and noradrenergic innervation for neocortex. The present study was undertaken to determine whether the brainstem was also a source of cholinergic input to neocortex.

Peroxidase anti-peroxidase (PAP) immunohistochemical staining with monoclonal antibodies shown to be specific to choline acetyltransferase (ChAT) revealed intensely positive neurons in the deep tegmental grey of the monkey mesencephalon. The region containing these neurons corresponds mostly to the nucleus cuneiformis and probably also overlapped with the nucleus tegmenti pedunculopontinus. The ChAT positive neurons were arranged into a compact lateral and a diffuse medial subdivision. ChAT positive neurons were seen to penetrate into adjacent fiber bundles.

Animals were injected with DFP. Tissue subsequently processed for AChE showed an intense reaction in these deep tegmental neurons. This contrasts sharply with the weak AChE positivity in other non-cholinergic but AChE containing nuclei such as the substantia nigra. We found that in the cuneiformis nucleus, all ChAT-positive neurons were also AChE-rich and that there were no AChE-rich neurons that lacked ChAT.

In order to demonstrate that neurons of this nucleus project to cortex, we examined the brainstem of several monkeys which had received cortical HRP injections (i.e. cingulate, insula, areas 5 and 8). Retrogradely labeled neurons were observed in the same nuclear region that contained the ChAT and AChE rich neurons. In tissue processed for the concurrent demonstration of AChE and HRP, HRP labeled neurons in this nucleus were also AChE-rich. On the basis of tissue processed for the concurrent demonstration of ChAT and AChE, we concluded that these HRP labeled, AChE-rich neurons are also ChAT positive. Therefore, our findings demonstrated the presence of a cholinergic projection from the deep tegmental grey to many cortical regions. Compared to the cholinergic input from the nucleus basalis, this appears to be a rather minor projection. For example, in five cases with cortical HRP injections a semiquantitative analysis revealed a mean of 100 HRP-labeled neurons per case in this cholinergic nucleus of the tegmentum as compared to a mean of 2200 neurons per case in the nucleus basalis. However, the number of cell bodies that give rise to projections give little indication as to the number of cortical terminations. These findings indicate that the deep tegmental grey contributes an additional cholinergic input to neocortex. Supported by NIH grants NS 17661, 09211, and the Essel , the Whitehall and the Francis Ledder Foundations.

38.6 LOCALIZATION OF BOMBESIN-LIKE IMMUNOREACTIVITY IN THE SPINAL CORD AND SENSORY GANGLIA: IMMUNOHISTOCHEMICAL EVIDENCE FOR DUAL ORIGIN OF THE PEPTIDE IN THE SPINAL CORD. P. Panula*, M. Hadjiconstantinou*, H.-Y.T. Yang and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Indirect immunofluorescence and peroxidase/antiperoxidase methods were used to study the localization of bombesin-like immunoreactivity (BN-LI) in the spinal cord and sensory ganglia of the rat. Rhizotomies were carried out at lower lumbar and sacral level to study the possible contribution of sensory fibres in the BN-LI of the spinal cord. Transection of the spinal cord at midthoracic level was performed to find out the extent of immunoreactive fibres originating from higher level of the CNS. Fresh 8 or 15 μm thick cryostat sections of formalin fixed spinal cords and spinal sensory ganglia were used for immunohistochemistry. The specific bombesin antiserum produced in rabbits was diluted 1:1000 and incubation was carried out for 48 hours at 4°C. The swine anti-rabbit antiserum and the PAP complex were diluted 1:50 and incubations were carried out at room temperature for 30 min. When immunofluorescence was used the sections were incubated with rhodamine-conjugated swine anti-rabbit antiserum, diluted 1:40 for one hour. Consecutive sections were incubated with bombesin antiserum preabsorbed with different amounts of bombesin or substance P. Bombesin was found to block the staining whereas substance P had no effect. In radioimmunoassay procedure our bombesin antiserum cross reacted with substance P less than 0.1%. In normal spinal cord, BN-LI was found in superficial layers of the posterior horn, around the motoneurones of the anterior horn and around the central canal in varicose fibres and terminal-like structures. After rhizotomy, the staining in the posterior horn diminished considerably whereas the staining of the anterior horn was unchanged. Transection of the spinal cord left the staining in the posterior horn unchanged and the terminals in the anterior horn were seen as well. Bombesin-like immunoreactivity was found in spinal ganglion cells but the number of immunoreactive cells was lower than the number of substance P-immunoreactive cells as revealed by a specific substance P antiserum. When consecutive sections were stained with the two antisera different cells were stained. We conclude that a bombesin-like peptide is present in the spinal ganglion cells which project to the posterior horn of the spinal cord. The rest of the BN-LI in the spinal cord may be derived from spinal interneurones or enter the spinal cord from the sensory ganglia between the transection and rhizotomy levels. It appears that the sensory ganglion cells that contain BN-LI are different from those that store substance P.

38.7 IMMUNOCYTOCHEMICAL LOCALIZATION OF MONOAMINE OXIDASE-B IN ADULT RAT BRAIN AND EARLY QUAIL EMBRYOS. P. Levitt[1], J.E. Pintar[2], G.D. Maxwell[3] and X.O. Breakfield[4]. [1]Sect. Neuroanatomy & Dept. Human Genetics, Yale Univ. Sch. Med., New Haven, CT 06510. [2]Dept. Anat. & Med., Mt. Sinai Sch. Med., New York, NY 10029. [3]Dept. Anatomy, UConn Health Ctr. Farmington, CT 06032

The mitochondrial enzyme monoamine oxidase (MAO) is responsible for deamination of many biogenic amines. Two types of MAO activity (MAO-A and MAO-B) can be distinguished on the basis of substrate affinities and drug sensitivities, and are mediated by two distinct enzymes. Pharmacological analysis has revealed differential distribution of MAO-A and MAO-B activities in the adult rat central nervous system (CNS). In addition, developmental analysis in whole quail embryos has demonstrated the presence of both MAO-A and MAO-B activities. In general, these approaches have not been useful in elucidating the specific cellular distribution of the two enzymes. Using an antiserum raised against pure bovine MAO-B (enzyme from Dr. James Salach), we have performed immunocytochemical analysis to localize MAO-B specifically in the adult rat CNS and in quail embryos at different developmental stages.

MAO-B immunoreactivity is present in two major CNS cell classes in the rat: astrocytes and serotonin-containing neurons. This localization was confirmed in double immunofluorescence experiments using antisera to glial fibrillary acidic protein or serotonin. Both protoplasmic and fibrillary astrocytes are immunoreactive throughout the brain, whereas oligodendrocytes are MAO-B negative. In addition, cells in regions lacking a blood-brain barrier, such as the circumventricular organs, are MAO-B positive. Ependymal cells lining the ventricles also stain for MAO-B. Catecholamine-containing neurons, such as those in the substantia nigra and locus coeruleus are MAO-B negative, although astrocytes in these regions are heavily stained. The presence of MAO-B in certain cell types may be related to the regulation of biogenic amine entry into the CNS and control of amine levels.

Quail embryos at 2-5 days of development contain MAO-B immunoreactive cells in the truck regions previously shown to contain or accumulate biogenic amines (including serotonin). These structures include the yolk sac, notochord, ventral neural tube and bilaterally symmetric cell populations near the dorsal aorta (likely the primary sympathetic chains). In addition, immunoreactivity is observed at the junction of the dermatome and myotome and within scattered cells throughout the embryo that may be neural crest derived. These results, combined with previous embryonic MAO activity measurements suggest that MAO-B may help to regulate amine levels in specific cell populations during early embryonic development.

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38.8 LOCALIZATION OF AROMATIC L-AMINO ACID DECARBOXYLASE IN NON-MONOAMINERGIC NEURONS OF THE RAT SPINAL CORD. C.B. Jaeger, G. Teitelman, V.R. Albert*, D.H. Park, T.H. Joh and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

In the central nervous system (CNS), the enzyme aromatic L-amino-acid decarboxylase (AADC) is believed to be contained only in these systems of neurons which synthesize and store serotonin (5HT), and the catecholamines. In the periphery, however, AADC has been found in some endocrine cells (e.g. pancreatic islet, Teitelman et al., Proc. Natl. Acad. Sci. USA 78:5225, 1981) that do not synthesize monoamines. We sought to determine whether non-monoaminergic neurons which contain AADC exist in the CNS.

Immunocytochemical staining using highly speicific antibodies against AADC, tyrosine hydroxylase (TH), phenylethanolamine-N-methyltransferase (PNMT), and 5HT revealed the presence of cells containing AADC in the spinal cord of neonatal and adult rats. In contrast, TH, PNMT, and 5HT could not be localized in perikarya similar to AADC positive cells. Cells containing AADC were found primarily in area X of Rexed surrounding the central canal. They had a variable distribution at different levels of the spinal cord. Most AADC positive cells were juxtaposed to the ependymal cells of the central canal. They had small round to oval cell bodies and several short processes. Electron microscopic examination of AADC cells stained by preembedding immunocytochemistry showed that AADC containing cells had features common to neurons: typical asymmetric synapses were found between dendrites of AADC cells and small axonal boutons. Axon terminals containing pleomorphic vesicles abutted the soma of AADC positive cells. With few exceptions, AADC containing neurons of the spinal cord extended at least one of their processes into the lumen of the central canal.

This is the first demonstration of a population of AADC containing neurons in the CNS which do not synthesize monoamines. The observations raise the question of transmitter identity in these neurons. Moreover, their morphology make the spinal AADC cells good candidates for "spinal cerebrospinal fluid (CSF) contacting neurons" that are present in numerous vertebrates including mammals (Vigh and Vigh-Teichman, Int. Rev. Cytol. 35:189, 1973). Conceivably, like spinal CSF contacting neurons, the spinal AADC neurons possess a pathway of communication via the CSF to other areas of the central nervous system.
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38.9 ANALYSIS OF SYNAPTIC INPUT TO VASOPRESSIN (VP) NEURONS IN THE PARAVENTRICULAR NUCLEUS (PVN). A.J. Silverman and A. Hou-Yu*. Depts. of Anat./Cell Biol. and Neurol., Columbia Univ., P&S, New York, N.Y. 10032.

The PVN is recognized as a regulatory center of autonomic function. Although information has been obtained in the last few years on the cytoarchitectonic organization of the PVN and its efferent projections, relatively little is known about the synaptic input to the various cell types. We now report our observations on the synaptology of immunocytochemically identified VP neurons using ultrastructural immunocytochemistry alone or in combination with 3H-norepinephrine (NE) radioautography. A monoclonal antibody to vasopressin (MAVP) (Clone III D-7) that does not cross-react with either oxytocin or arginine vasotocin was produced (Hou-Yu et al., J. Histochem. Cytochem., in press). This antibody was used to identify the VP cells. Adult rats were perfused with either lysine periodate paraformaldehyde, 2% glutaraldehyde or 2% glutaraldehyde, 1% paraformaldehyde with 0.002% $CaCl_2$ and 3% sucrose. Vibratome sections were cut, treated with 0.2% Triton X 100 for 20 min and then exposed to the MAVP for 48hrs. Sections were next incubated in a second antibody conjugated to HRP followed by DAB and H_2O_2. These sections were dehydrated and embedded EPON 812 for EM. Reaction product was present in the cytoplasm of the somata, dendrites and axons. A major synaptic input is observed on all portions of the VP cell. For dendrites and somata, synapses occur on spinous processes as well as on non-spinous regions of the plasma membrane. Both symmetrical and asymmetrical membrane specializations were observed and the presynaptic element contained either clear spherical, elongate or pleomorphic vesicles. Occasionally terminals with dense core vesicles and without apparent membrane specializations were seen. On axons in the median eminence the majority of synapses were asymetrical with clear, round vesicles. To determine if any of these terminals contained norepinephrine, we infused 100 uCi of 3H-NE into the ateral ventricle or 10 uCi directly into the tissue. At approriate times thereafter the animals were perfused with fixative nd tissue processed for EM immunocytochemistry as above. Thin ections were coated with Ilford nuclear emulsion according to he method of Kopriwa and exposed for up to 3 mos. Silver grains ver axon profiles and immunocytochemical reaction product were bserved in the same thin sections demonstrating that the techniues are compatible. This approach makes possible the qualitaive and quantitative analysis of the NE input to VP neurons. upported by AM 20337, a grant from the Whitehall Fnd (AJS) and n MDA fellowship (AH-Y).

38.10 IMMUNOCYTOCHEMISTRY OF OLFACTORY TUBERCLE AND OLFACTORY CORTEX. R.A.E. Bakay*, L.E. Westrum, A. Hendrickson (SPON: J.S. Lockard). Depts. Neurological Surgery, Biological Structure, and Opthalmology, Univ. of Washington, Seattle, WA 98195, and J.Y. Wu, Dept. Cell Biology, Baylor College of Medicine, Houston, TX 77030

The olfactory tubercle has been shown to have a very strong reactivity to certain transmitter enzymes, especially glutamic acid decarboxylase (GAD) but less is known of the pattern in the adjacent and interconnected olfactory cortex. Also since recent receptor-binding studies in these areas implicate opiate receptor binding here, the comparative examination of this region for enkephalin reactivity would be particularly useful. Olfactory tubercle (OT) and the adjacent prepyriform cortex (PYR) are being studied immunocytochemically with a variety of specific antibodies to transmitters enzymes. Included are commercially obtained antisera to met-enkephalin and GAD antisera prepared according to the protocol of J.Y. Wu. With light microscopy, GAD reactivity in OT was seen predominantly in deeper layers, including layer III and with especially positive accumulations adjacent to the Islets of Calleja and protruding into the scallops of layer II cells. Somewhat less distinct reactivity, granules and fibers were seen in layer I, especially near the pia. A few large cells deep in layer III show slight reactivity in colchicine injected material. The PYR shows definite reactive fibers and terminals throughout layer I but somewhat concentrated immediately under the lateral olfactory tract and deep layer I nearer the cell bodies. No reactive cells were seen in colchicine material. Enkephalin reactivity was striking in OT but complementary to GAD with a heavy distribution throughout layers I, II and upper III, avoiding to a great extend the GAD positive deeper areas. Islet of Calleja and their GAD positive cores were not enkephalin reactive. PYR had reactive fiber and terminals in upper layer I, and deeper in layer III. Reactive cells were rare in colchicine injected material. The results show a GAD and enkephalin-related reactivity which is complementary but with some areas of overlap.

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39.1 EFFECTS OF HYPOTHALAMIC PERIVENTRICULAR AND AMYGDALAR LESIONS ON EPISODIC GROWTH HORMONE AND THYROTROPIN SECRETION AND SOMATOSTATIN. L. C. Terry, W. R. Crowley, C. Lynch*, C. Longserre*, R. Free* and N. Petersen*. Depts. of Neurol. & Physiol., U. of Michigan and VA Hosp., Ann Arbor, MI 48105 and Dept. of Pharmacol, U. of Tennessee, Memphis, TN 38163.

Somatostatin (SRIF) inhibits growth hormone (GH) and thyrotropin (TSH) secretion in the rat, and passive immunization with SRIF antiserum elevates plasma GH & TSH. Earlier studies (W.R. Crowley & L.C. Terry, Brain Res. 200:283, 1980) have shown that lesions of the hypothalamic periventricular nuclei (HPV) and medial-basal amygdaloid nuclei (AMYG), which contain high concentrations of SRIF neurons, deplete SRIF in the median eminence (ME). The present study assessed the involvement of these SRIF systems in the regulation of basal episodic GH and TSH secretion by placing discrete lesions in the HPV and AMYG.

Two experiments were performed using freely-behaving, chronically cannulated, male albino rats (300-400g) kept on a constant light-dark cycle (12-12h, lights on at 600h) with food and water ad lib. In one experiment, bilateral electrolytic anodal lesions (2mA x 10sec) were placed in the HPV at the level of the paraventricular nucleus. In a second experiment, bilateral thermocoagulation lesions (55°C x 1min) were placed in the AMYG. Blood samples were removed from animals every 15min for 5.5h (beginning at 1000h) 2 weeks postoperatively. Animals were then decapitated and their brains were removed rapidly and frozen. Individual brain nuclei were microdissected and assayed for SRIF (radioimmunoassay) and protein content (Lowry method). Plasma concentrations of GH and TSH were determined by a double antibody radioimmunoassay. Only animals bearing correctly placed lesions were evaluated.

HPV lesions significantly reduced SRIF levels in the ME (227.8 ± 48.1 vs 557.3 ± 22.7 pg/ug protein) and arcuate (18.9 ± 5.9 vs 42.4 ± 8.7) and anterior HPV nuclei (6.6 ± 1.1 vs 31.4 ± 10.5). AMYG lesions caused a smaller but significant reduction in ME SRIF (554.2 ± 56.3 vs 750.1 ± 81.7). Neither the total amount of GH secreted (area under curve, trapezoidal method) nor plasma GH peaks, troughs or interpeak intervals were altered significantly by HPV or AMYG lesions. However, AMYG lesions caused a significant elevation in the total amount of TSH secreted (195,897 ± 7192 vs 152,087 ± 11,650 ng/ml x min) and mean plasma TSH levels (508 ± 16 vs 380 ± 18 ng/ml). HPV lesions did not significantly alter plasma TSH levels.

These results suggest that (1) SRIF neurons in the HPV and AMYG do not have a significant function in regulation of basal episodic GH secretion, and (2) AMYG but not HPV SRIF perikarya are involved in regulation of basal TSH secretion.

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39.2 GROWTH-INDUCING HIPPOCAMPAL TRANSECTIONS AFFECT LIMBIC AND GASTROINTESTINAL SOMATOSTATIN AND LIMBIC CHOLINE ACETYLTRANSFERASE CONCENTRATIONS IN HAMSTERS. K.T.Borer,S.Segal*,A.I.Vinik*&B.Shapiro*. Departments of Physical Education and Internal Medicine,University of Michigan,Ann Arbor,MI 48109.

Growth-inducing transections of dorsal hippocampus are associated with significant depletions of serotonin and norepinephrine from hippocampus and cerebral cortex (Borer,K.T. et al Brain Res.Bulletin 4:239,1979).To characterize further the neurochemical and peptidergic circuits responsible for suppression of somatic growth in adult hamsters, we transected bilaterally dorsal hippocampus in 18 adult female hamsters (Borer,K.T. et al.,Neuroendocrinology 29:22,1979),sham-operated 17 controls, and measured somatostatin and choline acetyltransferase concentrations in cerebral cortex,hippocampus,septum,median eminence,gastric fundus, antrum, and pancreas on day 14 of the experiment. To verify that cuts were effective in facilitating somatic growth, animals were weighed daily, and a blood sample was collected on day 14 for determination of hamster growth hormone by a homologous radioimmunoassay. After an initial weight loss, hippocampal cuts induced a significant ($p < 0.05$) increase in ponderal growth rate, and were associated with significantly higher concentrations of growth hormone in the serum (54.2 ± 7.9 vs 7.8 ± 1.6 ng/ml, $p < 0.01$). Other significant ($p < 0.05$) differences were as follows:

	SOMATOSTATIN (pg/mg)		CHOLINE ACETYLTRANSFERASE (pM/mg)	
	Hippocamp.cuts	Controls	Hippocamp.cuts	Controls
Hippocampus	198.7±18.1	299.5±42.1	522.9± 76.8	3105.8± 100.5
Septum	178.8± 9.8	167.5±13.5	5086.6±191.6	4651.5± 192.3
Antrum	52.4±10.0	29.4± 3.2		
Pancreas	98.0±17.4	184.3±22.0		

Significant depletion of somatostatin and choline acetyltransferase from hippocampus, and an apparent accumulation of both substances in the septum, suggest that hippocampal transection may be damaging somatostatinergic and cholinergic projections from septum to hippocampus.Significant shifts in gastroenteropancreatic somatostatin concentrations suggest a possible involvement of this peptide in the control of nutrient entry during lesion-induced acceleration of growth in adult hamsters.

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39.3 HIGH AFFINITY ALDOSTERONE RECEPTOR OF RAT BRAIN BINDS GLUCOCORTICOIDS AS WELL AS MINERALOCORTICOIDS. K. Beaumont and D.D. Fanestil*. Dept. of Medicine, Univ. of California, San Diego, La Jolla, CA 92037

We have investigated the steroid specificity and regional distribution of the high affinity cytoplasmic aldosterone (ALDO) receptor recently identified in rat brain (Anderson and Fanestil, 1976). ^{3}H-ALDO binding to adrenalectomized rat brain cytosols was measured by the dextran-coated charcoal method at concentrations varying from 0.2 to 50 nM. Biphasic Scatchard plots of saturation data were obtained. At 4°C, in the presence of Mo^{++} to stabilize receptors, affinities of the two sites for ^{3}H-ALDO were 0.28 ± 0.23 nM and 18.0 ± 2.5 nM, with densities of 33.0 ± 4.4 fmole/mg pro and 221 ± 29 fmole/mg pro, respectively (mean ± SEM, n=6). At these concentrations, ^{3}H-ALDO did not bind detectably to corticosteroid binding globulin (CBG) of adrenalectomized rat serum. However, ^{3}H-corticosterone (B) did bind to CBG under these assay conditions with an extrapolated affinity of 0.3 nM at infinite protein dilution. Therefore, prior to specificity studies, CBG was eliminated from cytosol preparations of perfused rat brain by selective precipitation of cytosol receptors with 36% NH_4SO_4. Less than 2% of ^{3}H-B binding to NH_4SO_4 precipitates of perfused brain cytosols was to CBG. The specific glucocorticoid RU 26988 (Mogiulewsky and Raynaud, 1980) was used to block low affinity ^{3}H-ALDO binding. Specificity of steroids for displacing ^{3}H-ALDO from the high affinity receptor under these conditions is DOC > 9αfluoroF > B > ALDO > Cortisol > spironolactone > Dex > TRIAM. Addition of 0.6% dialyzed serum to cytosol, an amount similar to that contaminating unperfused brain homogenates, altered the apparent specificity of this site by sequestering unlabeled DOC and B. The specificity in the presence of CBG was ALDO > DOC >> B, similar to the mineralocorticoid receptor of kidney. Specificity of the low affinity ^{3}H-ALDO binding site was similar to that of the glucocorticoid Type II steroid receptor. Regional distribution of the two receptors was determined in presence of 10^{-8}M RU 26988 to block low affinity binding or 10^{-8}M spironolactone to block high affinity receptors. Both receptors are at highest density in hippocampus and least dense in hypothalamus. Outside of these areas, the regional distribution of the two receptors is not correlated. The high affinity receptor is most concentrated in limbic areas (amygdala, septum, temporal cortex, striatum) while the low affinity receptor is at high density in cerebellum and cortex. These studies indicate that the high affinity aldosterone receptor of rat brain may bind either corticosterone or aldosterone under conditions used for autoradiographic and in vitro experiments. Which endogenous steroid activates this receptor under physiological conditions is not known.

39.4 OPIATE RECEPTOR MECHANISMS OF PROLACTIN AND GROWTH HORMONE RELEASE IN THE RAT. K. Spiegel, I.A. Kourides* and G.W. Pasternak. Dept. of Neurology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021.

Serum prolactin and growth hormone levels are markedly increased by opiates and opioid peptides. The subpopulation(s) of opiate binding sites which mediates this release has not been well established. Recent work suggests the existence of sites which selectively bind morphine-like drugs, termed mu_2 sites, and other sites which preferentially bind enkephalins, called delta sites. Recent studies also suggest an additional, higher affinity site, termed mu_1, which binds both opiates and enkephalins very potently. Naloxazone is an opiate antagonist which selectively blocks mu_1 sites irreversibly.

Rats had chronic intravenous cannulae placed. Dose response studies demonstrated that maximal prolactin release required lower doses of morphine than those needed for the maximal growth hormone response. Groups of rats (n=6) were treated with either naloxone or naloxazone (50 mg/kg,iv). After waiting 24 hours to permit elimination of naloxone and nonirreversibly bound naloxazone, baseline levels were drawn, all animals given morphine sulfate (10 mg/kg,iv), and additional samples taken at 15, 30, and 45 minutes. Peak prolactin levels in the naloxone group, seen at 15 min., were 152 + 32 ng/ml, quite similar to untreated controls (119 + 13 ng/ml; n=4). Blockade of mu_1 sites by naloxazone treatment dramatically lowered the peak prolactin levels, also seen at 15 min., to 29 + 8ng/ml ($p<0.003$). In contrast, peak growth hormone values in naloxazone treated animals (648 + 161 ng/ml) were increased 250% relative to the naloxone treated group (260 + 89 ng/ml; $p<0.10$). These results suggest different receptor mechanisms for the opiate modulation of the two hormones. Mu_1 sites appear to mediate the morphine-induced release of prolactin but not growth hormone.

39.5 STUDIES ON THE INHIBITORY ROLE OF MORPHINE ON THE RELEASE OF DOPAMINE INTO THE HYPOPHYSIAL PORTAL VASCULATURE AND ON THE SYNTHESIS OF DOPAMINE BY TUBEROINFUNDIBULAR NEURONS. M.J. Reymond* and J.C. Porter (SPON: M. Stewart). Cecil H. and Ida Green Center for Reproductive Biology Sciences, Depts. of Obstetrics and Gynecology and Physiology, Univ. of Texas Health Sci. Center at Dallas, Dallas, TX 75235.

A consequence of treatment of rats as well as humans with morphine is hyperprolactinemia due to the release of prolactin from the anterior pituitary gland. This effect of morphine treatment appears to be due, at least in part, to an inhibitory action of morphine on the tuberoinfundibular dopaminergic neurons of the hypothalamus. In the present investigation, we evaluated the efficacy of morphine, administered intracerebroventricularly to ovariectomized adult rats, on the release of hypothalamic dopamine into the hypophysial portal vasculature. In addition, we sought to ascertain the extent to which the suppression of the release of hypothalamic dopamine was due to the inhibition of the synthesis of dopamine in the tuberoinfundibular neurons, as evaluated by the accumulation of dihydroxyphenylalanine (DOPA) in the median eminence of rats given 3-hydroxybenzylhydrazine (NSD 1015), a centrally acting inhibitor of the activity of aromatic L-amino acid decarboxylase. During the 60 min following the intracerebroventricular administration of 60 ng of morphine sulfate, a 90% reduction in the rate of release of hypothalamic dopamine into hypophysial portal blood occurred. A dose-related decrease in the rate of release of dopamine into the portal vasculature was observed between 1 ng and 60 ng of morphine sulfate. Regardless of the quantity of morphine sulfate (1 ng to 500 ng) given to the animals, the concentrations of norepinephrine and epinephrine in hypophysial portal plasma and femoral arterial plasma remained unchanged, and no evidence was obtained for hypothalamic release of norepinephrine or epinephrine. The efficacy of morphine on the release of dopamine into the hypophysial portal blood was not associated with an equal efficacy of the drug on the synthesis of dopamine in tuberoinfundibular neurons. No effect of morphine was observed on DOPA accumulation in the median eminence of NSD-treated rats that had received 50 ng of morphine sulfate intracerebroventricularly, and only a 50% reduction was observed in the accumulation of DOPA in the median eminence of rats given 500 ng of morphine sulfate. These findings are illustrative of a discordance in the effect of morphine on the amplitudes of the release and the synthesis of hypothalamic dopamine, and they may be suggestive of a direct influence of morphine on the mechanisms of release of dopamine from the tuberoinfundibular neurons into the hypophysial portal vasculature.

39.6 ELEVATED GLUCOSE UTILIZATION IN NEUROHYPOPHYSIS AND SUBFORNICAL ORGAN OF BRATTLEBORO RATS LACKING VASOPRESSIN. M. Kadekaro, P.M. Gross, H.H. Holcomb§, L. Sokoloff and J.M. Saavedra§§. Laboratory of Cerebral Metabolism, Biological Psychiatry Branch§, and Laboratory of Clinical Science§§, National Institute of Mental Health, Bethesda, Maryland 20205.

Brattleboro rats with hereditary diabetes insipidus (DI) are characterized by an abnormally high water intake and correspondingly high output of hypo-osmolar urine. Despite the fact that these animals cannot elaborate vasopressin, the hypothalamo-neurohypophysial system is hypertrophied and presents morphological characteristics compatible with enhanced metabolism.

In an attempt to characterize the functional significance of these morphological alterations we have employed the 2-[^{14}C]deoxyglucose quantitative autoradiographic technique in the conscious Brattleboro rat. Long-Evans (LE) rats were used as controls. Results were obtained from 3-6 month old male rats fed normal rat chow and tap water ad libitum. Water intake was monitored in the individually caged animals. DI rats (n=6) consumed 92±4 ml/100 g body weight/24 h. LE rats (n=6) drank 11±0.3 ml/100 g body weight /24 h. The table below shows that glucose utilization was selectively increased in the subfornical organ and in the neurohypophysis of DI rats. The hypothalamic nuclei responsible for the secretion of vasopressin in normal animals were not metabolically different in Brattleboro rats. Values for glucose utilization (in µmoles/100 g/min) of structures listed are expressed as means ± SEM of 6 rats.

	LE	DI	
Subfornical organ	51 ± 3	73 ± 3	* $p < 0.001$
Paraventricular n.	62 ± 4	58 ± 4	
Supraoptic n.	60 ± 2	62 ± 4	
Suprachiasmatic n.	66 ± 4	61 ± 5	
Neurohypophysis	44 ± 3	88 ± 8	* $p < 0.001$

The finding that the glucose metabolic rate is elevated in the subfornical organ is interesting inasmuch as this structure mediates drinking in response to high plasma levels of angiotensin II. DI rats are known to have increased plasma concentrations of sodium and angiotensin II. The mechanisms and significance of the elevated glucose utilization in the neurohypophysis are not known.

39.7 GENETIC DIFFERENCES IN CNS ANDROGEN AND ESTROGEN RECEPTORS IN MICE. Neal G. Simon* and Richard E. Whalen (SPON: M. J. Weiss). Long Island Research Institute, Health Sciences Center T-10, SUNY, Stony Brook, NY 11794.

Evidence for a relationship between genotype and hormone binding macromolecules in the CNS has been provided through studies of androgen-insensitive (Tfm) male rats, mice and humans. With this mutation an 85-95% reduction in androgen receptor (AR) levels is seen in comparison to unaffected males. Whether there are genetically determined differences in CNS cytosol receptor proteins for androgens and estrogens among non-mutant males, however, remains to be determined. We addressed this question by examining diethylstiboestrol (DES) and dihydrotestosterone (DHT) binding in hypothalamic-preoptic-septal sections from orchiectomized CD-1, CF-1, and CFW outbred mice.

A modification of the Chamness, et al. (Brain Res., 1979) hydroxylapatit assay was used for 6-point saturation analyses. Cytosols were incubated for 3 or 4 hr with varying concentrations of ^{3}HDES (.04-1.84 nM) or ^{3}HDHT (.078-6.28 nM) at 0-4° C with or without a 100-fold excess of the appropriate unlabeled hormone.

Scatchard analyses of the data revealed that for both hormones there were differences among the strains either in the number of binding sites (B_{max}) and/or in the avidity of the binding (K_d). In the ER assays, CFW males had the highest B_{max} (12.81 fmol/mg protein), followed by CD-1 (9.22 fmol) and CF-1 (6.8 fmoles)males. The apparent K_d in CF-1 males (.14 x 10^{-10}M) was lower than that observed in the other strains (both .32 x 10^{-10}M). AR levels were again greatest in CFW males (B_{max} = 10.36 fmol), followed by CF-1 (9.68 fmol) and CD-1 (8.13 fmol) males. The apparent K_d values for DHT binding to AR were 1.04 x 10^{-10}M (CFW), 2.89 x 10^{-10}M (CF-1), and 2.36 x 10^{-10}M (CD-1).

The results are in reasonable accord with other studies of AR and ER in mice. The detection of differences among genotypes in apparent K_d and B_{max} values suggest that these factors may contribute to strain differences in hormonal sensitivity

39.8 EFFECTS OF POSTERIOR HYPOTHALAMIC LESIONS ON THE ONSET OF MENARCHE AND TIME OF THE FIRST OVULATION IN THE FEMALE RHESUS MONKEY. Ei Terasawa, Michael D. Loose*, John J. Noonan* and Thomas E. Nass*, Wisconsin Regional Primate Research Center, Neuroscience Training Program, University of Wisconsin, Madison, Wisconsin 53715-1299.

The effect of experimental lesions in the posterior hypothalamus on the onset of menarche and on the time of first ovulation was examined in a non-human primate. Bilateral lesions were made through a thermister electrode in the posterior hypothalamus with the aid of X-ray ventriculography in female rhesus monkeys (n=7) at 18 months of age. Electrode tip diameter and tip exposure were 0.7 mm and 1.0mm, respectively, and an electrode tip temperature of 73°C was maintained for 1 min. A total of eight animals of a similar age served as controls and sham controls. All animals were caged individually and examined daily for vaginal bleeding and sex-skin color changes. In addition to the weekly blood drawing for monitoring the changes in hormonal parameters, body weight and nipple size measurements were obtained periodically. The time of the first ovulation was determined by laparoscopic observation of the newly formed corpus luteum and by the values of circulating progesterone. After confirmation of the 2nd ovulation, animals were autopsied and the location and extent of lesions were histologically determined. The bilateral lesions were approximately 2-3 mm in diameter and overlapped the center line of the hypothalamus. In all animals lesions included the premammillary area and the posterior nucleus, while the arcuate nucleus was entirely spared. In most cases lesions encroached upon the ventromedial nucleus and the dorsomedial nucleus rostrally, or the mammillary nucleus caudally or both.

Posterior hypothalamic lesions advanced the age at menarche (22.2±1.3 months, p=0.014, Mann-Whitney U test) and the first ovulation (40.7±2.7 months, p=0.047) when compared to those of control animals (menarche 30.3±3.1; the first ovulation 51.6±4.2 months). The body weight at menarche of lesioned animals (2.6±0.1 kg) was smaller (p=0.047) than that of controls (3.1±0.2kg), but the body weight at the first ovulation of lesioned animals (4.4± 0.3kg) was not different from that of controls (4.3±0.2kg). Hormonal and physical changes during maturation in lesioned animals occurred earlier than those in controls; i.e., an increase in circulating estradiol and growth in nipple size prior to menarche and the first ovulation, and the growth spurt prior to the first ovulation in lesioned animals not only began earlier, but also attained mature levels several months earlier than control animals.

Therefore, lesions in the posterior hypothalamus induced true precocious puberty in the female rhesus monkey. (Supported by NIH grants RR-00167, HD11355, HD15433.)

39.9 NEURAL PATHWAYS MEDIATING LUTEINIZING HORMONE SECRETION IN MALE MICE. Arthur Coquelin and Roger A. Gorski. Department of Anatomy and Laboratory of Neuroendocrinology of the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

As in most mammals, male mice release luteinizing hormone (LH) in abrupt, intermittent episodes. These dramatic, all-or-nothing elevations of circulating LH occur spontaneously or as a reflexive response to the presence of a female (Coquelin and Bronson, Endocrinology 106: 1224, 1980). Is there a single system of neurons that underlies both the intrinsic and reflexive patterns of episodic LH release in mice? As an initial approach to this question we examined LH secretion in mice after hypothalamic deafferentation. We expected that the spontaneous episodes of LH release would not be affected by either anterior (retrochiasmatic) or complete deafferentation of the basal hypothalamus, but that LH responses to social stimuli might be blocked by such surgery. Sexually inexperienced, individually housed, adult CF-1 males received either anterior deafferentation (n=10), complete deafferentation (n=9), or sham surgery (n=6) using a 28 ga Halasz-type stereotaxic knife. All males were implanted with intra-atrial cannulas one week later. Sequential blood samples (30 µl) were withdrawn at 5 min intervals beginning 5-6 days after cannulation; plasma LH was measured by radioimmunoassay. Blood was collected over the 6 hr period before through 1 hr after placing an ovariectomized female into the males' cages. All the control males and 8 of 10 anteriorly deafferented males exhibited 1-2 spontaneous episodes of LH release. Following exposure to females the anteriorly deafferented group demonstrated rapid rises in plasma levels of LH that were indistinguishable from those of the control group. Among the males that responded (6/10 anterior deafferentation vs. 4/6 sham; $X^2 = 0.37$, $p>0.50$), comparable peak values of plasma LH were attained ($\bar{x} \pm$ S.E., anterior deafferentation = 221 ± 49 ng/ml, sham = 248 ± 39 ng/ml; $F(1,8) = 0.16$, $p>0.70$). Neither spontaneous pulses nor hormonal responses to females typical of normal males were obvious in the completely deafferented group. Instead, irregular yet prominent fluctuations of basal LH occurred at approximately 30 min intervals and masked potential individual elevations of hormone following exposure to females. Analysis of variance with repeated measures revealed no overall increase of plasma LH after the social stimulus ($F(1,8) = 0.01$, $p>0.90$). Our results suggest that one neural input mediates the intrinsic as well as the reflexive pattern of LH release in male mice and that it originates outside the medial basal hypothalamus. These afferents may converge upon the median eminence along a rostral-to-caudal, lateral-to-medial projection. Supported by NRSA HD06160 to A.C. and NIH HD00182 to R.A.G.

39.10 COMPARISON OF UPTAKE AND RAPID RELEASE OF DOPAMINE (DA) FROM MEDIAN EMINENCE (ME) AND STRIATAL (S) SYNAPTOSOMES. Karen A. Gregerson*, Pierre Drapeau*, and Michael Selmanoff (SPON: Bruce K. Krueger). Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201.

Release of preaccumulated ^{3}H-DA from crude synaptosomal preparations (P_2 fraction) from rat ME and S was examined using a rapid filtration technique. Synaptosomes were prepared from comparable masses of tissue (10-15 mg) and run in conjunction for comparison between the tuberoinfundibular and nigrostriatal DA neurons. Synaptosomes were preloaded by incubation in 0.1µM ^{3}H-DA for 30 minutes at 30°C. At this concentration, uptake of ^{3}H-DA was about 2-fold greater into S than into ME synaptosomes, consistent with the differences in uptake kinetics between ME and S reported by Anunziato and coworkers (Neuroendo. 31:316, 1980). Addition of 5×10^{-7}M desipramine, a norepinephrine (NE) reuptake blocker, to the incubation medium, resulted in 20% inhibition of ^{3}H-DA uptake into ME synaptosomes, which may be accounted for by the percentage of NE terminals in the catecholamine population of the ME. Desipramine had no effect on ^{3}H-DA uptake into S synaptosomes, a comparatively NE-poor tissue. ^{3}H-DA release over short time intervals (1-10 seconds) was measured in 2-3 samples/mg tissue with a rapid filtration method using solutions containing 0.1% BSA, which we found to be necessary for stabilizing the synaptosomes. High K^+-induced release of ^{3}H-DA from ME synaptosomes was Ca^{++}-dependent and occurred in two phases. A "fast" phase of release occurred during the initial second of depolarization [rate constant of release (k_{rel}) = 0.012 sec^{-1}] and was followed by a leveling-off of release during the next 9 seconds. The k_{rel} during the fast phase was 2-3 orders of magnitude greater than that previously observed for ^{3}H-DA release from ME utilizing superfusion techniques. The k_{rel} for the fast phase in S synaptosomes (see also Drapeau and Blaustein, Neurosci.Abstr.7:441, 1981) was much greater than that for ME synaptosomes, suggesting that the kinetics of DA release, as well as those for uptake, are markedly different between the ME and S. We are currently using this highly sensitive technique to examine the effects of prolactin, β-endorphin and NE on the release of ^{3}H-DA from ME and S synaptosomes.
(Supported by NIH grants NS-14611 and NS-16106. P.D. is a recipient of a MRC of Canada Fellowship.)

39.11 MONOCLONAL ANTIBODIES TO TYROSINE HYDROXYLASE FROM THE CORPUS STRIATUM OF THE RAT. J.F. Sissom* and J.C. Porter. Cecil H. and Ida Green Center for Reproductive Biology Sciences, Depts. of Obstetrics and Gynecology and Physiology, Univ. of Texas Health Sci. Center at Dallas, Dallas, TX 75235.

Tyrosine hydroxylase was purified from the corpus striatum of the rat brain according to the procedure of Vulliet et al. (Proc. Natl. Acad. Sci. U.S.A. 77: 92-96, 1980). This procedure involved precipitation with ammonium sulfate, column chromatography employing DEAE and hydroxylapatite, and sucrose density centrifugation. The specific activity of tyrosine hydroxylase, assayed according to the method of Foreman and Porter (J. Neurochem. 34: 1175-1183, 1980), was increased 100- to 200-fold by this purification procedure. Homogeneity of the final product was confirmed by disc gel electrophoresis using SDS-PAGE. The molecular weight of the purified enzyme was approximately 60,000, a value similar to that reported by Joh et al. (Proc. Natl. Acad. Sci. U.S.A. 75: 4744-4748, 1978) for tyrosine hydroxylase in the rat brain. The purified enzyme was used in the immunization of mice of the BALB/c strain. When the presence of antibodies in the serum of the mice was detected, the spleens of the animals were removed, and the spleen cells were fused with mouse myeloma cells (NS-1 cell line). The procedure of Kennett et al. (Monoclonal Antibodies. Hybridomas: A New Dimension in Biological Analyses, Plenum Press, New York, 1980, pp. 368-369) was followed in the hybridization. An ELISA technique was used to identify hybridomas that secreted antibodies to tyrosine hydroxylase. Clones, identified as TH-1 and TH-6, of two antibody-secreting hybridomas were isolated in soft agar and propagated in cell culture. Clonally derived cells were injected intraperitoneally into mice, and monoclonal antibodies were harvested from the ascites fluid. To evaluate the specificity of TH-6 antibodies, 10 mg of immunoglobulins isolated from ascites fluid was covalently conjugated to 1 g of Sepharose 4B gel. A homogenate of rat corpus striatum tissue was prepared, and aliquots of the homogenate were subjected to affinity chromatography using TH-6 immunoglobulin-Sepharose 4B or glycine-Sepharose 4B, which served as the control. After chromatography on a TH-6 immunoglobulin-Sepharose 4B affinity column, the total activity of tyrosine hydroxylase in the homogenate was reduced 98% compared to the control. These results are supportive of the view that TH-6 antibodies are directed against tyrosine hydroxylase. On the basis of results obtained using assays specific for subclasses of mouse immunoglobulins, it would appear that these monoclonal antibodies are of the IgM class.

39.12 SLOW WAVE FIELD POTENTIALS IN THE RAT BRAIN DURING PUBERTY AND THE ESTROUS CYCLE. J. F. Masken and R. J. Morgan. Dept. of Physiology and Biophysics, Colorado State University, Fort Collins, CO 80523.

Chronic stainless steel electrodes were implanted in the amygdala (AMY), medial preoptic area (POA), and arcuate nucleus (ARC) of both prepubertal and adult normal female Sprague-Dawley rats. Slow wave field potentials were recorded daily from these areas simultaneously for the first 10 min of each half-hr from 7:30 h until 14:10 h throughout the onset of puberty and during the course of the estrous cycle. Recorded analog data was analyzed by cross-correlation analysis for the amount of correlation and direction of signal flow between pairs of electrodes (i.e. AMY-POA, AMY-ARC, POA-ARC), and for conduction times between pairs of sites.

The correlation of slow wave activity for AMY-POA in both prepubertal and adult rats was higher than that of AMY-ARC and POA-ARC with AMY-ARC being the lowest. The correlation for all three combinations, i.e. AMY-POA, AMY-ARC, and POA-ARC was lower in the prepubertal animal. The change in the level of correlation appeared gradually and prior to vaginal opening in the case of AMY-ARC and POA-ARC, but the correlation for AMY-POA increased rather abruptly and right at first estrus. The correlation for all three combinations in adult cycling rats was lowest during proestrus. Also, signal traffic from AMY to the POA lasts longer and takes longer en route during the "critical period" on the day of proestrus than at other times of the cycle, implying a change in signal pathway at this time. Other changes seen in the direction of signal flow (in which area does the signal originate) could be of significance with respect to daily fluctuation in GnRH and LH release.

40.1 EPENDYMAL NEUROGENESIS IN ADULT FEMALE CANARIES. Steven A. Goldman* and Fernando Nottebohm (SPON: D.R. Griffin). The Rockefeller University, New York, N.Y. 10021.

Previous reports have demonstrated the neuroplasticity of the songbird vocal control nucleus HVc. In male canaries this nucleus varies in volume seasonally, in concordance with seasonal song acquisition. The adult female's HVc expands in response to systemic testosterone administration; new dendritic outgrowth may comprise some of this observed increase in size, as has been previously shown for the vocal control nucleus RA. We became interested in the possibility of neuronal proliferation in the adult HVc, as a possible basis for both the gonadally-induced changes in gross HVc volume and the seasonal learning of song.

Sixteen intact adult female canaries were injected i.m. with ^{3}H-thymidine, 50 μCi every 8 hr over a 2 day period. Some birds were given testosterone implants, at varying times (0-18 days) before thymidine treatment, while others were given cholesterol implants before thymidine. The birds were killed 5 weeks after hormone implantation and their brains processed for autoradiography. All birds showed considerable numbers of labelled neurons, glia, endothelia and ependymal cells in and around HVc; little labelling was found elsewhere. Cholesterol and testosterone treated birds had similar neuronal labelling indices, which ranged from 1.8-4.0 % in HVc. Since all of these females had intact ovaries, we have not excluded a gonadal influence on neuronal recruitment, S-phase or survivability. In fact, four gonadectomized adult female canaries, subsequently treated with ^{3}H-thymidine, showed labelling indices that were depressed with respect to those of the intact females.

In order to better identify labelled HVc cells, two thymidine treated birds were prepared for electron microscopic analysis, and processed for combined light autoradiography and ultrastructural analysis of adjacent ultrathin sections. Cells tentatively identified as neurons in 1μm sections were confirmed as such ultrastructurally. We also determined the origin of the thymidine incorporating neurons, by sacrificing two thymidine treated females within 24 hr after their thymidine injections, thereby precluding significant migration of the newly born labelled cells. Analysis of these brains revealed no cells of neuronal morphology present in HVc, but a very heavily labelled ependyma overlying HVc. We tentatively conclude that neuronal precursors exist in the HVc ependyma, which incorporate tritiated thymidine during the S-phase preceding their mitosis; after division, these cells migrate into (and to some extent beyond) HVc. This ependymal neurogenesis occurs in the absence of exogenous testosterone and seems to be a normally occurring phenomenon in intact untreated adult females.

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40.2 CONNECTIVITY AND KINETICS OF NEURONS BORN IN ADULTHOOD. Fernando Nottebohm and Steven A. Goldman*. The Rockefeller University, New York, N.Y. 10021.

We have noted in the preceding abstract the existence of ependymal neurogenesis in the adult female canary HVc. In an effort to more precisely elucidate the functional significance of the new HVc neurons, we have studied (1) the patterns of connectivity of these new neurons, (2) the extrapolated turnover of HVc with time, based on the kinetics of HVc neuronal recruitment, and (3) the relative pattern of neurogenesis in adult male canaries.

In a study of the connectivity of the new HVc neurons, two testosterone-implanted adult female canaries were pretreated with 60 μCi injections of ^{3}H-thymidine, twice daily for one week; after a one month hiatus these birds were given iontophoretic injections of the retrograde tracer HRP into two HVc projection nuclei, area X and RA. These nuclei each receive projections from HVc, thereby allowing the HRP to label those subclasses of HVc neurons projecting to each nucleus. The birds were sacrificed 24 hr later and their brains processed for combined HRP histochemistry and thymidine autoradiography. Preliminary data suggest that thymidine incorporating, newly born neurons may not retrogradely label with HRP, indicating that the new HVc neurons probably do not project to either of HVc's two principal targets, RA or area X. It is possible that the new cells project to another, as yet undescribed, target nucleus, but we believe it more likely that they serve as local interneurons within HVc.

We have determined that neurons are added to the adult female's HVc at the rate of approximately 1% per day of the total HVc neuronal pool. However, if the new cells do not include projection neurons, as our HRP data would seem to suggest, then a subclass of interneurons within HVc would account for all of the new neurons, indicating a recruitment much greater than 1% per day within this restricted subpopulation.

In a separate study we examined the possibility that the normal adult male canary might show HVc neurogenesis. Twelve one-year-old male canaries were injected for a two week period with ^{3}H-thymidine (30 μCi i.m., b.i.d. x 14 days) and sacrificed at varying intervals afterwards. Their brains were removed and processed for autoradiography. Analysis of these brains showed a considerable number of labelled, neuron-like cells in HVc; ultrastructural analysis of these cells is now being performed. We tentatively conclude that the adult male canary exhibits neurogenesis, presumably similar in nature to that of the adult female.

This research was supported by PHS grants 5R01MH18343 and S07 RR07065.

40.3 RECRUITMENT OF ADDITIONAL SYNAPSES INTO A BRAIN NETWORK TAKES EXTRA BRAIN SPACE. Timothy J. DeVoogd, Barbara Nixdorf* and Fernando Nottebohm. The Rockefeller University, New York 10021

Adult female canaries treated with testosterone (T) develop male-like song. This hormone treatment also induces an enlargement of telencephalic nuclei involved in song control. An earlier study showed that at least part of this enlargement was due to growth of dendrites. It was inferred at that time that this growth added to the network space controlling song. For this to be true, extra dendritic length would have to correspond to a net gain in number of synapses. In the present study, we test this hypothesis.

Six adult female canaries were given Silastic implants of crystalline testosterone following the fall molt. All began singing about two weeks later and gradually improved to the level of sustained, stereotyped song previously observed in T-treated females. Four treated females and five untreated control females were perfused for electron microscopy six weeks after hormone treatment. The brains were sectioned at 100u. Each slice was lightly counterstained and photographed. Slices containing nucleus robustus archistriatalis (RA) were alternately processed either with osmium tetroxide for conventional electron microscopy or with ethanolic phosphotungstic acid (EPTA) for selective staining of synapses. RA was then disected out of the slices and embedded in Epon. One T-treated female proved inadequately fixed and is not considered further. Synapses in EPTA-stained tissue were counted in medial, central and lateral regions of RA in sections 0.2u thick using dark field light microscopy (from G. Vrensen, personal communication). Since synaptic density did not vary between the regions sampled, these counts were pooled for later analyses. Ultrathin sections of the conventionally prepared tissue were stained with lead citrate and uranyl acetate. Synapses were counted in nine micrographs (32,400X) taken from the central region of each RA.

As shown in the table RA is more than 80% larger in the T-treated than in the control females. The density of synapses as measured by either technique, varies only slightly between treatment groups. This study is currently being replicated. We conclude from the present sample that T-induced song requires a substantially larger synaptic network than that of a non-singing female--obtained by extending RA.

	T-treated	Control
N	3	5
Synapses per $100u^2 \times .06u$	15.3	15.5
Synapses per $100u^2 \times .2u$	58.0	63.2
RA size x mm^3	.137	.074

40.4 RETINAL AND TECTAL GROWTH IN THE TELEOST FISH, HAPLOCHROMIS BURTONI. R. D. Fernald and J. Presson, Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

Teleost fish grow throughout their lifetime and consequently the nervous system adds cells and generates new synaptic connections during adulthood. For example, in the teleost H. burtoni, a 50% increase in body length is associated with a 33% increase in lens diameter (eye size) and a 100% increase in brain weight. H. burtoni offers significant advantages for analysis of the consequences of this post-embryonic growth of the nervous system. First, H. burtoni relies heavily upon its well developed visual system for behavioral interactions which are crucial to its survival. Second, growth of males in this species depends critically upon social interactions. Thus male growth can be controlled so that animals of any desired size and growth rate can be made available.

Previous studies of growth in lower vertebrates (Xenopus, goldfish) have suggested that retino-tectal synaptic partners must change to allow the growing retina to be mapped onto the growing tectum without disturbing the regularity of the retino-tectal map. This suggestion stems from the fact that the retina grows by adding annular rings of cells but the tectum grows in a "U" shaped pattern, adding few cells to the rostral pole while adding many cells to the caudal pole. Since cells are being added to the temporal retina but not to its tectal projection site, the rostral tectum, changing retino-tectal connections seems necessary.

To analyze retinal and tectal growth patterns in H. burtoni, 3-H thymidine was injected intraperitoneally to label the DNA of actively dividing cells. All types of retinal cells were labeled in a ring around the perimeter of the retina, and rod nuclei were labeled across the retina as reported previously. Although the temporal retina has a higher cell density than other regions at all ages, cells appeared to be added uniformly around the retina. This represents an additional constraint on retino-tectal growth in H. burtoni, since maintenance of the region of high density must be achieved without differential cell addition. Cell addition in the tectum of H. burtoni appears to be "U" shaped. Few labeled cells are found in the rostral tectum but the caudal tectum is heavily labeled. Analysis of growth after varying survival times will allow a comparison of the temporal and spatial dynamics of tectal cell addition with those of retinal cell addition. Supported by a grant from the Whitehall Foundation.

40.5 IMMUNOLOGICAL PRIVILEGE IN THE BRAIN WITH RESPECT TO FUNCTIONAL BRAIN GRAFTS. W.J. Freed,* H. E. Spoor, D. Sachs and R.J. Wyatt.* Adult Psychiatry Branch, NIMH, St. Eliz. Hosp., Wash. D.C. 20032; Immunology Branch, National Cancer Institute, NIH, Bethesda, Maryland 20205

Partial restoration of function following damage to certain brain nuclei in animals has recently been accomplished through transplantation of brain tissue. For example, fetal substantia nigra (SN) or adult adrenal medulla transplanted to the brain lateral ventricles partially restores dopaminergic function in the caudate nucleus following SN lesions. Much of this work has employed random-bred rat strains, such as Sprague-Dawley, in which homograft rejection would be expected to occur. These grafts may therefore survive because of the "privileged" status of the brain as a site for transplantation. To rule out the possibility that matching at the major histocompatibility complex (MHC) was responsible for this graft acceptance, we have studied survival and rejection of brain grafts in genetically-defined rat strains.

Rats of the inbred Fisher 344 (F344) strain were used as graft recipients. Donors were either syngeneic F344 rats or Brown Norway (BN) rats, having an MHC difference. Intraventricular SN allografts from BN donors were, in most animals, found to survive for at least four months and in some cases reduced lesion-induced rotational behavior. There was no unusual degree of macrophage infiltration, and the grafts produced catecholaminergic fibers which penetrated into the host (F344) brain.

Several months subsequent to the implantation of BN or F344 grafts, skin patches derived from the tails of either F344 or BN donors were grafted to the skin of the F344 recipients. Syngeneic F344 skin grafts were not rejected, while BN skin grafts were rejected in a mean of 12 $\pm$ 1.6 (mean $\pm$ SEM) days in animals with BN brain grafts, and 14 $\pm$ 1.6 days in animals with F344 brain grafts. This difference was not statistically significant ($T_{(13)}=0.8$, $p=0.44$). However, rejection of BN skin grafts was associated with disappearance of the BN brain grafts. No surviving BN brain graft tissue was found in any of the eight animals that had rejected a BN skin graft, while F344 skin grafts did not adversely affect BN brain grafts.

These findings, in keeping with the original observations of Medawar (Br. J. Exp. Pathol., 29:58, 1948), suggest that the privileged status of the brain as a site for transplantation can be abrogated by sensitization through another route. This is consistent with aberrant central processing of the immune stimulus (Kaplan, H.J. and Stevens, T.R., Transplantation, 19:302, 1975) or with an interruption in the afferent arm of the immune system. Thus brain grafts can survive and function across MHC differences, but fail when the host is sensitized by extracerebral antigenic stimulation.

40.6 RESPONSE PROPERTIES AND SOMATOTOPIC ORGANIZATION OF DORSAL HORN NEURONS FOLLOWING NERVE LESIONS IN THE CAT. L. M. Pubols. Dept. of Anatomy. The Medical College of Pennsylvania, Philadelphia, PA 19129.

It has been reported (Devor & Wall, J. Comp. Neurol. 199:277, 1981) that chronic sciatic and saphenous nerve lesions lead to striking alterations in dorsal horn somatotopic organization. It was the purpose of the present study to identify the population(s) of neurons responsible for this change by examining the receptive field properties of single neurons in chronically deafferented dorsal horns. Seven adult cats with lesions of the right sciatic and saphenous nerves were studied at survival times of 6-12 hr (1), 14 days (2), and 45-67 days (4). Responsiveness ipsilateral to acute (6-12 hr) deafferentation was greatly diminished. Results from chronic lesion animals, which were similar at all survival times, have, therefore, been compared with data from intact dorsal horns of 10 other animals. The right L6 dorsal horn was mapped mediolaterally in transverse rows of dorsoventral electrode penetrations spaced 200μm apart. The most medial penetration containing any responsive neurons was marked in each row by ejection of horseradish peroxidase from the electrode tip, and by using the same electrode as a marker 200μm lateral to this penetration. All receptive fields ipsilateral to the lesions were proximal to the ankle. The median location of the most medial active penetration in 18 rows of penetrations in chronic lesion animals was 71% of the distance from the medial to the lateral boundaries of the dorsal horn, vs. 86.5% of that distance for the most medial penetration with RF's proximal to the ankle in intact dorsal horns ($p < .02$, Mann-Whitney U; ranges = 25-95% vs. 68-98%). Some clearly aberrant somatotopy was observed. However, a silent zone in the medial dorsal horn was always found in the lesion group. A high proportion of single neurons ipsilateral to the lesions (71%, n = 21) showed convergence of input from both hair and high threshold mechanoreceptors, and many had large receptive fields. For 52% of single neurons the region of skin responding to hair movement was surrounded by a larger area responding only to skin compression. The response properties of neurons in the present study are similar to those of some classes of spino-cervical tract and postsynaptic dorsal column neurons (Brown & Franz, Exp. Brain Res. 7:231, 1969; Fyffe et al. Neurosci. Abstr. 7:611, 1981). Data from the present study provide only partial confirmation of the results of Devor and Wall, who found: (1) proximal receptive fields across the entire width of the dorsal horn within 28 days of sciatic and saphenous nerve lesions; and (2) uniform mechanical thresholds throughout the receptive fields of neurons ipsilateral to deafferentation.

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40.7 THE AREA 3b REPRESENTATION OF THE HAND IN OWL MONKEYS REORGANIZES AFTER INDUCTION OF RESTRICTED CORTICAL LESIONS. W. A. Jenkins, M. M. Merzenich, J. M. Zook, B. C. Fowler and M. P. Stryker. Coleman Laboratory, Depts. of Physiol. and Otolaryngol., UCSF, San Francisco, CA 94143.

In earlier studies, we demonstrated that transection of the median nerve or amputation of fingers results in a progressive reoccupation of the deafferented cortical zone representing these skin surfaces in Areas 3b and 1 in adult owl and squirrel monkeys. The nature of this reorganization led to the conclusion that representational changes of the same kind should also occur following restricted central somatosensory system lesions.

To test that hypothesis, a restricted cortical lesion was effected by microcauterization of surface vessels within a limited sector of the hand representation in Area 3b in adult owl monkeys. Following induction of the lesion, the representation of the hand was immediately mapped in detail, to functionally delineate the boundaries of the lesion and to precisely define the skin sector whose representation was lost consequent from it. One monkey was again mapped six days later; the boundaries of the lesion and the surviving overt representation were very similar to those defined immediately after making the lesion. By contrast, monkeys remapped 1 1/2 to 4 months later were found to have profoundly reorganized hand representations, with a re-emergence of topographic representations of skin surfaces formerly represented in the territory of the lesion. The locations and topographics of the representations of skin surfaces previously mapped over the cortical region surrounding the lesion were also significantly (sometimes dramatically) altered. The observed reorganizational changes: 1) Probably occurred at the thalamocortical or cortical level; 2) indicate that reorganization occurs with detection of the limits of the damaged cortex through the recovery process; and 3) probably underlie the relatively rapid functional recovery seen after limited cortical damage in this region in man. They further underscore the fact that cortical map structure is dynamically maintained in adult primates.

Further studies are being directed toward determination of the distances over which such reorganizational changes occur, and to determine whether this reorganization process constitutes the principal basis for recovery from stroke. (Supported by NIH Grant NS-10414 and Hearing Research Inc.)

40.8 ISOLATION VERSUS GROUPED HOUSING IN RATS: DIFFERENTIAL EFFECTS OF LOW DOSES OF HEROIN IN THE PLACE PREFERENCE PARADIGM. T. Hunt*, S. Schenk*, L. Colle*.(SPON:L.Switzman)Centre for Research on Drug Dependence, Montreal, Quebec, Canada.

Male long Evans rats were reared from weaning (21-23 days) either in isolation or in groups of four for 40 days. Animals were then individually introduced to a testing apparatus consisting of two distinct chambers. A modified place preference paradigm was used consisting of 3 phases: (1) An habituation phase (4 days) during which rats were allowed free access to the entire test apparatus for 15 min. periods daily; (2) A conditioning phase (4 days) during which rats were confined to their non-preferred side for 15 minutes each day immediately following subcutaneous injection of heroin HCl; (3) Test phase (1 day) during which rats were again allowed free access to the testing chamber following injection of vehicle. The difference in time spent on the conditioned side during habituation and test periods was determined. The group-reared rats showed maximal effects at the lowest dose of heroin whereas the same magnitude of drug effect was attained only at the highest dose used in the isolated rats. This differential sensitively to heroin in the place preference paradigm is discussed in terms of the modification of behavioral effects of opiates by environmental influences.

40.9 PERIPHERAL NERVE STIMULATION ENHANCES RECOVERY OF FUNCTION FROM NEUROLOGICAL DEFICITS IN HUMANS.

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Electrical subcutaneous nerve stimulation (SCNS) of radial, median, and saphenous nerves abolished clonus temporarily in subjects with spasticity according to a double-blind study (Science, 216, 203 (1982). Unilateral stimulation suppressed clonus on the contralateral side, and stimulation of the nerves in the wrist suppressed ankle clonus indicating the mechanism mediating the effect must be centrifugal inhibition. After repeated administration of SCNS, subjects can be free of clonus for months.

SCNS also produced improvement of intention tremor in subjects with cerebellar damage. Unilateral SCNS produced ipsilateral improvement. These results, taken together, indicate that SCNS exerts its effect centrally and raises the intriguing possibility that SCNS produces long-lasting changes in neuronal circuitry.

41.1 ADRENAL GLANDS PARTICIPATE IN THE REDUCED FEAR RESPONSE IN SPONTANEOUSLY HYPERTENSIVE RATS. J.E. LeDoux, A. Sakaguchi and D.J. Reis, Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

The behavorial response to aversive stimulation is reduced in spontaneously hypertensive rats (SHRs) when compared with strain-matched Wistar-Kyoto controls (WKYs) in nocioceptive tests and in tests of conditioned fear (LeDoux et al., Fed. Proc. 41: 1364, 1982; Hypertension, in press). That endogenous opiate systems might play a role in these strain differences is indicated by the observation of increased opiate receptor binding in the SHR brain (Martucci and Hahn, Endo. Res. Commo., 6: 291, 1979) and by the fact that treatment of SHRs with the opiate antagonist naloxone enhances both the hypoalgesia and the reduced fear response (LeDoux et al., Fed. Proc., 41: 1364, 1982). The adrenal glands have long been implicated in fear responses (Weiss et al., Science, 163: 197, 1969; Moyer, J. Genetic Psychol., 92: 17, 1958) and adrenal medullary cells contain enkephalin (Wilson et al., PNAS, 77: 4364, 1980). If the adrenal glands contribute to the reduced fear response in SHRs, adrenalectomy should enhance the response; if adrenal opiates are involved, naloxone should have no effect in adrenalectomized SHRs. Classical conditioning (30 trials) was used to establish fear reactions to environmental stimuli. The unconditional stimulus (US), an electric footshock (0.5 sec, 1.5 mA), was delivered at the termination of the conditional stimulus (CS), a pure tone (10 sec, 800 Hz, 80 db). Conditioned fear behavior was assessed the next day by measuring the duration of immobilization ("freezing") during a 300 sec presentation of the CS. Freezing was assessed in: (a) untreated SHRs (n = 10) and WKYs (n = 10); (b) SHRs subjected to bilateral removal of the adrenal glands (n = 6) or sham operation (n = 6); (c) adrenalectomized SHRs treated with saline (2 ml/kg, ip; n = 5) or naloxone (10 mg/ml/kg; n = 5). The duration of freezing (in sec) was less ($p<.01$) in SHRs (171 ± 12) than in WKYs (275 ± 12), thus confirming the strain difference in fear behavior (LeDoux et al., Fed. Proc., 41: 1364, 1982). Freezing in adrenalectomized SHRs (261 ± 12) was greater ($p<.01$) than in sham operates (174 ± 15), indicating that the adrenal glands might play a role in the reduced fear response. Treatment of adrenalectomized SHRs with naloxone reduced ($p<.01$) the duration of freezing (153 ± 18) relative to adrenalectomized SHRs treated with saline (255 ± 15), indicating opiate stimulation rather than blockade. These data demonstrate that adrenalectomy in SHRs eliminates the strain difference in conditioned fear and that treatment with naloxone reverses the effects of adrenalectomy. We conclude: (a) the adrenal glands play a role in the reduced fear response in SHRs; (b) adrenalectomy may alter the effects of naloxone at opiate receptors.

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41.2 Rapid Development of Tolerance to Exogenous Cholecystokinin on Satiety-Related Behaviors

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Cholecystokinin octapeptide (CCK_8) reduces food intake in fasted rats, mice, sheep, pigs, monkeys, and humans.[1] CCK reduces exploratory and social behaviors, initiating the behavioral sequence associated with satiety.[2,3] The present study investigated the ability of chronic CCK_8 administration to produce long lasting changes in body weight and satiety-related behaviors. Alzet osmotic minipumps containing CCK_8 1.0 µg/kg/hr, 0.1 µg/kg/hr, caerulein 1.0 µg/kg/hr, 0.1 µg/kg/hr, or saline were intraperitoneally implanted. Daily food intake and body weight were recorded over the two week infusion. Behavioral responses to a challenge dose of CCK_8 were evaluated during chronic infusion and after removal of the minipump. No significant change in body weight was detectable with any treatment at any time point. Time course of the exploratory behavioral responsiveness to the CCK_8 challenge indicated a rapid development of tolerance to chronic CCK within the first day of infusion. Exogenously administered CCK_8 appears to induce satiety more effectively by acute administration than by chronic infusion.

1. Morley, J. A. Life Sci. 30: 479-493, 1982. The ascent of cholecystokinin (CCK)-from gut to brain.

2. Antin et. al., J.C.C.P. 89:7: 784-790, 1975. Cholecystokinin elicits the complete behavioral sequence of satiety in rats.

3. Crawley et. al. Peptides 2:1: 123-129, 1981. Neuropeptide modulation of social and exploratory behaviors in laboratory rodents.

41.3 Hypothalamic(HYP), Pituitary and Plasma Levels of Pro-opiomelanocortin-Derived(POMC) Peptides During Feeding Stimulated by 2-deoxy-D-glucose(2DG) and Insulin(INS) Administration in Rats. J. Mark Davis*, Marie J. Gibson, and Dorothy T. Krieger, Div. of Endocrinology, Mount Sinai Medical Center, New York, NY 10029

In order to further investigate the possible relationship between β-endorphin(β-ep) and feeding stimulated by 2DG (400mg/kg) and INS(10 U/kg) injections(sc), we measured several POMC-derived peptides in the HYP, pituitary lobes, and plasma in 5 groups(n=8) of male rats studied 60 min post-SAL,-2DG, or -INS injection, or 150 min post-SAL or -INS injection. At 60 min food intake in INS was 3-fold higher, and in 2DG 10-fold higher, than in SAL group. Food intake in INS at 150 min was 4-fold higher than SAL, but equivalent to 2DG at 60 min.

Table 1: Plasma immunoreactive(IR)-peptides and corticosterone(B)

	IR-β-ep+		IR-ACTH+		B++	
	60′	150′	60′	150′	60′	150′
SAL	154±11	269±55	47.3± 3.5	80.2±12.2	2.5±0.8	2.7±0.8
2DG	488±43*	-	465.0±77.6*	-	33.1±2.4*	-
INS	264±68	178±30	128.0±19.4*	88.8±10.8	11.6±2.1*	7.5±1.1*

+ pg/ml; ++ µg/100 ml; * $p<0.05$ vs. saline control

Table 2: HYP,ant.(AP) and neurointermediate(NIL) pituitary peptide

		IR-β-ep		IR-ACTH		α-MSH	
		60′	150′	60′	150′	60′	150′
	SAL	.40±0.6	-	-	-	.27±0.6	-
HYP+	2DG	.31±0.5	-	-	-	.14±0.1*	-
	INS	.30±0.2	.36±0.5	-	-	.13±0.1*	.20±.03
	SAL	.09±.01	.09±.01	.14±.04	.19±0.3	-	-
AP+	2DG	.10±.01	-	.09±.01	-	-	-
	INS	.11±.01	.18±.05	.18±.05	.08±.01*	-	-
	SAL	.98±.21	1.19±.23	-	-	.87±.30	.51±.05
NIL§	2DG	1.06±.20	-	-	-	.62±.11	-
	INS	1.12±.10	1.19±.23	-	-	.66±.23	.84±.23

+ ng/mg wet wt.; § µg/mg wet wt.; *$p<0.05$ vs. saline

Increases in plasma IR-ACTH and B were greater following 2DG than INS. Only 2DG was associated with increases in plasma IR-β-ep. Both stimuli were associated with similar decreases in HYP α-MSH levels. No changes were seen in HYP IR-β-ep levels. The only change in pituitary IR-peptide levels was a decrease in IR-ACTH in the AP at 150 min following INS.

Further characterization of other forms of these peptides is in progress. These preliminary findings suggest that both 2DG and INS produce similar alterations in POMC-derived peptides in HYP, but quite different responses in plasma. The significance of these changes relative to reported differential naloxone effects on 2DG and INS feeding remain to be determined.

41.4 MODULATION OF CNS INSULIN RECEPTORS BY STREPTOZOTOCIN-INDUCED DIABETES: EVIDENCE FOR INDEPENDENT INSULIN RECEPTOR SYSTEMS IN THE CNS. R. J. Waldbillig*, D. J. Steel*, and M. S. Kappy* (SPON: T. Schoenfeld). Depts. of Psychology and Pediatrics, University of Florida, Gainesville, Florida 32611

Recent evidence indicates that the brain contains an insulin-insulin receptor system. Published reports have shown that neither CNS insulin levels nor insulin receptor binding in brain tissue are affected by diabetes or systemic hyperinsulinemia However, these analyses were performed on whole brain homogenates, and it is entirely possible that an analysis employing greater anatomical selectivity might reveal regional differences. This is most likely to occur in circumventricular areas such as the hypothalamus and area postrema, where it has previously been shown that blood-borne (pancreatic) insulin is capable of binding to neural tissue (Van Houten and Posner, Diabetologia, 20: 255, 1981) The purpose of the present work was to determine the effect of streptozotocin-induced diabetes on insulin receptors in the olfactory bulb, medial and lateral hypothalami, area postrema and liver.

Three weeks following intravenous streptozotocin (65 mg/kg) treatment, twenty male Long-Evans rats were decapitated,and the above mentioned areas assayed for insulin receptor binding by the method of Havrankova et al. (J. Clin. Invest. 64: 636, 1979).

The results of this work indicate that compared to non-streptozotocin treated controls, diabetic rats exhibited an increased insulin receptor binding in the medial and lateral hypothalamus, area postrema and liver. The extent of the hypothalamic "up-regulation" seems to be greater in the medial area than in the lateral. In contrast to its effects in the hypothalamus and area postrema, diabetes produces a "down-regulation" of insulin receptors in the olfactory bulb. These data are taken to indicate that unlike the olfactory bulb, circumventricular areas are exposed to pancreatic insulin and are "up-regulated" in its absence. The insulin receptors in the olfactory bulb may instead reflect levels of activity in an endogenous insulin system.

41.5 EVIDENCE FOR AN INSULIN-SENSITIVE HYPOTHALAMIC CIRCUIT CONTROLLING HEPATIC GLUCOSE PRODUCTION. G. E. Weider*, and R. J. Waldbillig* (SPON: G. M. Hope) Department of Psychology, University of Florida, Gainesville, Florida 32611

Recently evidence has been presented indicating that the brain contains an insulin and insulin receptor system that is independent of pancreatic insulin (Havrankova and Roth, Diabetologia 20: 268, 1981). However, there is also evidence that blood-borne insulin binds to neurons in the medial hypothalamus (Van Houten and Posner, Diabetologia 20: 255, 1981). The studies presented here examined the role of insulin receptors in hypothalamic function by investigating the relationship between the level of hypothalamic insulinization and plasma glucose levels.

The results of this work indicate that in non-diabetic free-moving rats 1.0 ul infusions of regular insulin (50-200 uU) into the ventromedial nucleus (VMN) produce a short latency, long-lasting and dose-dependent reduction in plasma glucose. Studies infusing ^{125}I-insulin indicate that this hypoglycemic effect is not the result of insulin "leaking" into the vasculature. Preliminary evidence also indicates that the hypoglycemic effect is not secondary to an increased pancreatic insulin release because an enhanced hypoglycemic effect can be produced in diabetic (streptozotocin treated) rats. More recent studies have revealed that blood samples drawn from the hepatic vein show a greater hypoglycemic effect than those drawn from the vena cava. This is interpreted as indicating that VMN insulin infusions produce a reduction in hepatic glucose output. Current work is using hepatic vagotomys to investigate the role of the autonomic nervous system in VMN-insulin induced hypoglycemia.

41.6 CHOLECYSTOKININ (CCK-8) SPECIFICALLY ANTAGONIZES OPIATE-MEDIATED ANALGESIAS. P.L. Faris*, B.R. Komisaruk, L.R. Watkins, and D.J. Mayer. Inst. Animal Behavior, Rutgers Univ., Newark, NJ 07102 and Dept. Physiol., Med. Coll. VA, Richmond 23298.

Since the effects of CCK are opposite those of opiates on feeding behavior and catecholaminergic activity, and CCK and opiates coexist anatomically, CCK may function as an endogenous opiate antagonist. We therefore examined the effects of CCK on opiate-mediated analgesia produced by environmental stimuli or morphine. Watkins, et al. (Pain (1981) suppl. 1: S263) have shown that hind paw (HP) and front paw (FP) shock produce non-opiate and opiate analgesia, respectively, on the tail flick test. Opiate analgesia was also produced using a classical conditioning paradigm (CCA) (FP shock: UCS; non-electrified grid: CS). Using these paradigms to elicit opiate-mediated analgesias, we found that CCK-8 (3 µg/kg IP) attenuated both FP shock-induced analgesia (FSIA) and CCA ($p<0.0001$). Morphine analgesia (10 mg/kg IP) was also attenuated by CCK-8 (5 µg/kg IP). Desulfated CCK-8 (des CCK) failed to antagonize FP FSIA and CCA. Additionally, the antagonistic effects of CCK-8 are specific to opiate analgesias, since HP (non-opiate) FSIA was not attenuated by CCK-8 in either intact or T2 spinalized animals. To determine the effective dose range and also the anatomical localization of CCK-8 action, we administered intrathecally (IT) 3.6, 20, 36, 200 and 360 ng of CCK-8 immediately prior to FP shock. Both FP FSIA and CCA were attenuated by 3.6 and 360 ng CCK-8 ($p< 0.0001$); the other doses were ineffective. Since 3.6 ng of des CCK did not affect FP FSIA ($p=0.17$) or CCA, but 360 ng des CCK did produce antagonism, only the effect of 3.6 ng CCK-8 appears to be specific to the sulfated form of CCK. At the 360 ng dose, the effect is apparently due to the gastrin-like properties of the sulfated and unsulfated forms of CCK-8, because an equimolar dose of secretin, which belongs to a different family of GI peptides, failed to antagonize FP FSIA. The effect of CCK-8 can not be explained by a hyperalgesic effect independent of an opiate interaction since 3.6, 36 and 360 ng IT CCK-8 did not affect baseline levels of pain responsiveness. These findings suggest that blockage of CCK-8 action may be effective in treating chronic pain, and an increase in CCK-8 action may mediate morphine tolerance. In addition, the satiation-producing effects of CCK-8 may be mediated through its anti-opiate properties. Supported by NSF Grant BNS78-24504 (BRK) and PHS DA00576 (DJM).

41.7 BEHAVIORAL EFFECTS OF CORTICOTROPIN-RELEASING FACTOR (CRF) AND RELATED PEPTIDES. D. R. Britton, [1]K. Lederis*, D. Hoffman*, [2]W. Vale and [2]J. Rivier*. Dept. Physiol., Sch. Med. Univ. New Mexico, Albuquerque, N.M., 87131, [1]Dept. Pharmacol., Univ. Calgary, Calgary. ALTA, CAN., [2]Peptide Biol. Lab., Salk Inst., La Jolla, CA.

In 1981, a 41 amino acid residue peptide of ovine hypothalamic origin was purified and synthesized (Vale et al., 1981). This peptide (referred to as CRF) is a potent stimulus to the release of ACTH and beta-endorphin from the anterior pituitary, both in vitro and in vivo. In addition, intracerebral ventricular (icv) administration of CRF produces activation of the sympathetic nervous system (Brown et al., 1982) and peripheral administration causes vasodilitation. Many of these effects are shared by the structurally related peptides, sauvagine (SA) and urotensin I (UT). Because of the potentially important role of CRF in mediating some of the endocrine responses to stress, we were interested in studying its behavioral effects. We hypothesized that CRF might produce behavioral expressions of the stress response just as it appeared capable of producing endocrine and sympathetic nervous system expressions of stress responding. We have previously shown (Britton et al., 1982) that icv CRF does produce behavior which mimics, in many ways, that observed in response to the stress of exposure to a novel environment. Specifically, fasted rats were given access to a single food pellet secured in the center of a novel open field and observed for 15 min. During this time, CRF treated rats (1.5 to 150 pmol/rat, icv) showed increased grooming, decreased food consumption, decreased duration of eating episodes as measured by the mean amount eaten per approach to the food pedistal, decreased rearing and decreased number of approaches to the food. The present data indicate that many of these behaviors are still observed in animals which have been habituated to the modified open field by several days of repeated exposure. Similar effects are seem upon icv administration of the related peptides SA and UT. These data indicate that the ability of CRF or related peptides to produce behavioral expressions of stress are not dependent upon the presence of external environmental stressful stimuli.

41.8 CONTRAVERSIVE ROTATION FOLLOWING B-ENDORPHIN, BUT IPSIVERSIVE ROTATION FOLLOWING MORPHINE MICROINJECTION IN RAT MIDBRAIN RETICULAR FORMATION. Y.F. Jacquet. Center for Neurochemistry, Rockland Research Institute, Ward's Island, New York City, 10035.

A unilateral microinjection of B-endorphin (2.9×10^{-9} mols) in the rat caudal midbrain reticular formation resulted in a late-onset episode of rotation consistently contraversive to the side of microinjection. A similar microinjection of morphine (2.6×10^{-8} mols) also resulted in a late-onset episode of rotation that was consistently ipsiversive to the side of microinjection. Met-enkephalin (1.7×10^{-8} mols), similar to B-endorphin, resulted in contraversive rotation (that was of immediate onset, but of short duration -- approximately 15 min). We previously reported (Jacquet, et al, Science, 1977) that the unnatural enantiomer of morphine, (+)-morphine, resulted in ipsiversive rotation at this site, indicating that the ipsiversive rotation was a non-stereospecific action of morphine. The present findings of opposing actions of B-endorphin and morphine in vivo parallel the in vitro findings previously reported (Jacquet, Science, 1980) in which morphine stimulated, but B-endorphin inhibited, the electrically-stimulated contractions of the rat vas deferens. In the present paradigm, ipsior contra-versive rotation may differentiate between the excitatory or inhibitory actions of opiates and opiate peptides. The former is non-stereospecific and not blocked by naloxone, while the latter is stereospecific and blocked by naloxone.

41.9 CORTICOTROPIN RELEASING FACTOR PRODUCES BEHAVIORAL ACTIVATION AND IMPROVES LEARNING IN RATS, G.F. Koob, M. Le Moal, F.E. Bloom, R.E. Sutton*, J. Rivier* and W. Vale. A.V. Davis CTR Behavioral Neurobiology and Peptide Biology Labs, Salk Inst. San Diego, Ca. 92138 and Lab. Neurobiologie des Comportements, Université de Bordeaux II, Bordeaux, France.

A 41 Amino acid peptide, with the ability to stimulate the secretion of corticotropin and β-endorphin, corticotropin releasing factor (CRF), has been examined for its ability to produce behavioral action. Male, albino Wistar rats were each equipped with a chronic cannula aimed above the lateral ventricle to allow acute intra-cerebroventricular (i.c.v.) injection of CRF. Rats were then well habituated to photocell activity cages (20 x 25 x 36 cm each with two horizontal infrared photocell beams across the long axis 2 cm above the floor). Several days later, the rats were rehabituated to the photocell cages for 90 min and then injected i.c.v. with 0.015, 0.15 and 1.5 nmoles of CRF corresponding to 0.1, 1.0 and 10.0 micrograms, respectively. CRF produced a dose-dependent increase in activity that was significant at the 0.15 and 1.5 nmole doses. Whereas saline injected rats rapidly went to sleep, CRF injected rats had high activity counts for a period lasting over five hours at the highest dose. Behavioral observation revealed a set of behaviors which appeared to reflect a general behavioral activation including elevated walking, grooming, rearing, and even climbing up and down the two mesh sides of the photocell cages. Behavior at the highest dose was characterized by more bizarre forepaw treading and repetitive locomotion. In contrast to these results, CRF injected i.c.v. at similar doses produced a dose-dependent decrease in activity in a novel open-field test. A dose of 0.15 nmoles of CRF, injected one hour before the test, significantly decreased outer square crossings and rearings and virtually eliminated inner square activity. Peripheral, subcutaneous injection of CRF did not produce any of these effects. CRF, injected in a dose of 0.15 nmoles (one hour prior to testing, for each of six days) significantly facilitated the learning of a visual discrimination in a Y maze. Here, rats were well habituated to the maze, and CRF injections began only after formal acquisition testing was begun. These results suggest that CRF can produce behavioral activation in rats that is dependent on the stressfulness of the situation, and that this activation can facilitate learning.

41.10 PHARMACOLOGICAL INDUCTION AND BLOCKADE OF LONG AXIS ROTATION IN RATS SUGGEST INVOLVEMENT OF NEUROPEPTIDES IN MINIMAL BRAIN DYSFUNCTION. A.K. Dua*, R. Bose* and C. Pinsky* (SPON: L.M. Wilson). Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada R3E 0W3.

The phenomenon of rotation in animals and human beings around their longitudinal axis is a rare one. Recently this phenomenon has been described in rats ("barrel rolling") after intracerebroventricular (ICV) infusion of vasopressin, luteinizing hormone releasing hormone, substance P, dynorphin, somatostatin and bradykinin. We observed this phenomenon unexpectedly in rats after ICV administration of bacitracin 200 µg. Rats (180-200 g) were prepared with indwelling cannulae implanted stereotaxically in right lateral ventricle. Just before or at the end of infusion most rats displayed long-axis rotation (LAR) in their cages. On a flat surface the rotation was propulsive, carrying the animal for distances of up to 70 cm. The rat is typically quiet at the start of rolling, then turns its head upwards halfway and extends the contralateral forelimb upward; LAR begins at this point. Rolling is initiated actively in the upper half of the body and is soon followed by hind limb involvement. This twisting motion thus leads to LAR in the entire body. During all stages of LAR the eyes are fixed and there is no nystagmus. The LAR response to ICV bacitracin is blocked by naloxone 5.0 mg kg^{-1} and amphetamine 7.5 mg kg^{-1}, given twenty min prior to bacitracin. Diazepam 5.0 mg kg^{-1}, i.p. blocks the response when given after the onset of LAR, as does phenytoin 1.0 µg ICV.

There is no known common structural moiety among those neuropeptides which produce LAR, and no common site of action has become evident. It is possible that excess release of neuropeptides, in patients displaying the LAR syndrome, somehow interacts with a number of different central mechanisms to produce the phenomenon of LAR. Since naloxone blocks bacitracin-induced LAR (this study) and atropine blocks somatostatin-induced LAR (Cohn and Cohn, 1975) the syndrome might represent a dyskinetic expression of generalized malfunction in multiple neurotransmitter pathways. Amphetamine blockade of LAR has several interesting clinical implications. The rolling could be an expression of hyperkinesia in animals, and this model may prove useful in exploring the juvenile hyperkinetic syndrome in minimal brain dysfunction, for which no good model had been described.
This work was supported by Medical Research Council (Canada), by Fellowship awards to AKD from Govt. of India and University of Manitoba and by a Manitoba Health Research Council Fellowship award to RB.

41.11 EFFECT OF THE PEPTIDE CYCLO(LEU-GLY) ON OPIATE-INDUCED DOPAMINERGIC SUPERSENSITIVITY. J.M. Lee*, R.F. Ritzmann, K.A. Steece*, and J.Z. Fields. Dept. of Physio. Biophys., Univ. of Illinois Med. Ctr., Chicago, Ill. 60612. and Dept. of Pharmacol., The Chicago Med. Sch., North Chicago, Ill. 60064.

Recent reports from our laboratories have indicated that the peptide, cyclo(Leu-Gly) (cLG), an analog of MIF(Pro-Leu-Gly-NH_2), administered prior to chronic exposure to opiates, prevents the development of both analgesic tolerance and some signs of physical dependence. The same peptide treatment also prevented the development of morphine-induced increases in certain physiological respones to the dopamine agonist apomorphine (APO). The present study investigated behavioral and physiological responses (stereotypy and hypothermia) to APO in an attempt to correlate these phenomena with neurochemical changes-specific (3H)-spiroperidol binding to dopamine receptors (DA-R)-occurring in the rat striatal and hypothalamic DA systems, respectively.

The experiments were performed following chronic opiate treatment with and without prior cLG administration. Changes in the parameters for binding of antagonists to DA-R have been documented (Ritzmann, et. al., Life Sci. 30:1573-80 (1982). With regard to interaction of agonist with the DA-R, the IC-50 curves for displacement of (3H)-spiroperidol by DA appeared to fit a biphasic, two site model in both striatal and hypothalamic control tissues. Pseudo-Hill coefficients were less than unity (0.7 and 0.5, repectively). The high affinity component of the DA inhibition curve, comprising about 20% of the total specific binding, had and IC-50 value of 1 x 10^{-7} M. Chronic opiate treatment led to a 10-fold decrease in the IC-50 value for the high affinity site (indicating an increase in affinity of DA for the DA-R) in both striatal and hypothalamic tissue. This increase in affinity was blocked by prior peptide administration. In parallel, cLG prevented the increase in APO-induced stereotypy and hypothermia which was observed in chronic opiate treated rats.

These data suggest that the ability of the peptide to block the development of some signs of physical dependence induced by opiates may involve the ability of the peptide to interfere with the opiate-induced changes in DA systems, particularly, with the DA receptor sites that recognize the agonist, dopamine.
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41.12 BOMBESIN EFFECTS ON THE MESOLIMBIC DOPAMINE SYSTEM OF THE RAT. D.W. Schulz*, P.W. Kalivas*, C.B. Nemeroff and A.J. Prange, Jr. (SPON: P. Morell). Neurobiology Program, Department of Psychiatry and Biological Sciences Research Center, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

Exploratory locomotor behavior in rats has been reported to be dependent upon intact dopaminergic mesolimbic pathways. Neurotensin, an endogenous neuropeptide, injected directly into the ventral tegmental area (VTA) produces an increase in exploratory behavior which is correlated with increased dopamine turnover in the nucleus accumbens and blocked by intracerebroventricular (icv) administration of haloperidol, a neuroleptic drug. Bombesin, a tetradecapeptide with neurotensin-like properties has recently been reported to increase spontaneous locomotor activity in rats when administered icv. The purpose of the present study was to determine whether bombesin increases locomotor activity by an action on this mesolimbic dopamine system.

Adult male Sprague-Dawley rats were stereotaxically implanted with injection cannulae whose tips were localized to the A_{10} nucleus of the VTA or the lateral ventricles. Photocell cages were used to assess locomotor and rearing behavior, and local concentrations of dopamine and dopamine metabolites (3,4-dihydroxyphenylacetic acid and homovanillic acid) were analyzed by high performance liquid chromatography. Bombesin doses ranged from 0.05 to 1.5 µg.

Intra-A_{10} injection of bombesin produced a prolonged dose-dependent significant increase in locomotor activity and a marked suppression of rearing behavior. However, these effects were not reversed by icv haloperidol, and no significant changes in dopamine turnover (as measured by DOPAC/DA ratios) were noted in terminal mesolimbic regions. Furthermore, icv bombesin was equipotent with intra-VTA bombesin in inducing these behavioral effects.

Peripheral pretreatment with the histamine or acetylcholine antagonists diphenhydramine or atropine (10 mg/kg) respectively, did not alter bombesin-induced locomotor activity.

These data support the view that bombesin can modulate spontaneous locomotor behavior in rats, but that its primary site of action is probably not on mesolimbic dopamine neurons.

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42.1 Learned Change in the Spinal Stretch Reflex (SSR): Persistence and Reversal. J. R. Wolpaw and R. F. Seegal., Center for Laboratories and Research, NY State Dept. Health and Depts. of Neurology and Anatomy, Albany Medical College, Albany, NY 12201.

Monkeys can change the amplitude of the spinal stretch reflex (SSR) without change in initial muscle length or background EMG activity (Wolpaw et al., NS Absts 7:249 (1981)). Change is evident in 5-10 days and continues over several weeks. To better define the features of this learned change in primate CNS function, we studied its persistence and reversibility.

Five monkeys were trained 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 within a given range, a brief force pulse extended the elbow 3-4°. SSR amplitude (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 was given 100ms after pulse onset. Under the SSR↑ or SSR↓ condition, reward was given only if SSR was greater (SSR↑) or less (SSR↓) than a criterion value. Data were obtained from each monkey for up to 1 year. Electrodes, background EMG, steady extension force, and the first 40ms of pulse-induced movement were stable throughout.

Control condition data were obtained from each animal for 10-50 days. SSR amplitude was stable throughout this period. Next, each animal was exposed to the SSR↑ or SSR↓ condition. SSR amplitude changed appropriately over 10-20 days and stabilized at a new level. Then each animal was exposed to one or more of the following four manipulations on one or more occasions (each occasion preceded by restabilization of SSR amp).

1) When the same condition was maintained, SSR amp remained stable (followed up to 50 days).

2) When the same condition was maintained but the criterion made harder, SSR amp changed appropriately by an additional increment with a time course similar to the initial change.

3) When the animal was removed from the task for a period of 4-30 days, SSR amp on task resumption was similar to that at task cessation; that is, learned change was preserved.

4) When the condition was reversed (SSR↑ to SSR↓ or visa versa), SSR amp changed appropriately with the usual time course.

These observations, that SSR change persists without continued performance and that further change or reversal of previous change occurs as slowly as initial change, suggest that SSR change may prove a technically approachable system for studying the neuronal and synaptic bases of a learned change in primate CNS function.

42.2 IS LONG-TERM POTENTIATION IN THE DENTATE GYRUS DEPENDENT ON AN ALTERATION IN THE PRESYNAPTIC TERMINAL ACTIVITY? B. R. Sastry, S. S. Chirwa*, J. W. Goh* and H. Maretic*. Neuroscience Research Laboratory, Department of Pharmacology, The University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

Potentiation of the hippocampal response that occurs after a tetanic stimulation of the inputs is long-lasting and has been implicated in processes such as learning and memory. The present investigations were undertaken to examine whether a presynaptic mechanism could account for this long-term potentiation (LTP). In vivo expts. were carried out on rats anaesthetized with a mixture of methoxyflurane, N_2O and O_2. Stimulating and recording electrodes were placed in dentate gyrus (DG) and in the ipsilateral entorhinal cortex (EC). In some expts., an extra stimulating electrode was placed in the non-terminal regions (NTR) of perforant path (PP). The field potential (FP) in the DG produced by a stimulation in EC and the antidromic field potential (AFP) in EC caused by a stimulation in DG or the NTR of PP were evoked at 0.2 H_z. In some rats, a seven-barrelled micropipette was positioned in the DG to antidromically activate EC neurones and to iontophoretically apply drugs. Tetanic stimulations (20 H_z, 30 sec), caused a post-tetanic reduction (by 30-80%) in the size of the AFP in EC produced by a stimulation in DG (24 of 26 expts.), but not by stimulation at NTR of PP (8 expts.). The FP in DG was facilitated (by 30-60%, 17 of 17 expts.). The time course of the increase in the FP in DG paralleled that of the decrease in the AFP in EC. The threshold for antidromic activation in DG, but not at the NTR of PP of 12 single EC neurones was increased by 30-60% during the post-tetanic period. Mn^{++} (50-200 nA) and Co^{++} (50-200 nA), when iontophoretically applied in DG, counteracted the post-tetanic reduction of the AFP in EC (Mn^{++}: 6 of 7; Co^{++}: 5 of 6 expts.). Calcium antagonists are known to interfere with the development of LTP. In vitro studies were conducted on transversely sectioned hippocampal slices of rats. In these expts. the PP was stimulated (0.2 H_z) to evoke a FP in DG. Glutamic acid diethylester, when applied to the slice preparation during the tetanic stimulation (100 H_z, 5 sec) in amounts that could completely block the evoked FP in DG (8-12 mM), did not prevent the onset of LTP (7 of 9 slices).

These results indicate that LTP in DG is associated with a Ca^{++}-dependent reduction in the excitability of presynaptic terminals and that LTP could be elicited even if the subsynaptic receptors were blocked during the tetanic stimulation of the input. It is, therefore, possible that LTP is caused by an enhancement in the presynaptic efficacy.

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42.3 DIFFERENTIAL FUNCTIONAL RESPONSE CORRELATES OF HIPPOCAMPAL CELL TYPES IN THE BEHAVING RAT. E.P. Christian*, M.O. West and S.A. Deadwyler* (SPON: P.B. Smith). Dept. of Physiol. and Pharmacol., Bowman Gray Sch. of Med., Winston-Salem, NC 27101

Numerous studies have correlated discharge patterns of hippocampal neurons with either the spatial features of an animal's environment, or alternatively to the performance of specific conditioned behaviors. We attempted to determine whether these two seemingly disparate functional roles are subserved by a common or by possibly different hippocampal neuronal subpopulations.

Seven Sprague Dawley rats were chronically prepared with a detachable microdrive system positioned above the dorsal hippocampal formation, allowing isolation of single units in the CA1 and CA3 subfields. Electrode position was verified by stimulation of the perforant path and commissural afferent fiber systems. Cells were further classified on the basis of spontaneous and evoked firing characteristics described by Fox and Ranck (Exp. Br. Res., 41: 399, 1981). Activity of each isolated unit was recorded in the following situations: 1) during performance of an auditory tone discrimination task (Deadwyler et al., Science 211: 1181, 1981) and 2) during traversal to find water at specific locations on a 61 x 61 cm square elevated platform.

The results are summarized in the table below:

	Correlates of Unit Firing	
	Spatial	Conditioning
Theta Cells	0/15	14/15
Complex Spike Cells	11/16	2/16

Complex spike cell firing was correlated with the animal's presence in a specific spatial location on the open platform, but not to the tone stimulus during conditioning. Theta cells, in contrast, showed a marked propensity to increase firing rate at the onset of the auditory conditioned stimulus, but showed no correlated discharge to distinct spatial aspects of the open platform. Firing activity of theta cells increased when the animal moved to any location on the platform.

This result is compatible with the hypothesis that there are two functionally distinct neuronal systems within the hippocampus. The fact that theta cell firing during conditioning resembled the discharge pattern previously reported for dentate granule cells in the same situation (West et al., Exp. Br. Res., 44: 287, 1981) suggests the possibility of a functional linkage between granule cells of the dentate gyrus and theta cells of the hippocampus proper. (Supported by NS 18288).

42.4 HIPPOCAMPAL PYRAMIDAL CELL ACTIVITY DURING TWO-TONE DISCRIMINATION AND REVERSAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE RESPONSE. T.W. Berger. Psychobiology Program, Departments of Psychology and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

Previous work has demonstrated that hippocampal pyramidal cells exhibit a learning-dependent increase in firing frequency during one-tone (i.e., sensory discrimination) conditioning of the rabbit nictitating membrane (NM) response (Berger, T.W. and Thompson, R.F., Proc. Nat. Acad. Sci., 75, 1978). Despite this fact, a consistent finding has been that bilateral hippocampectomy does not retard behavioral learning in such a paradigm (e.g., Solomon, P.R. and Moore, J. W., JCPP, 89, 1975). Recently, we have shown that bilateral hippocampectomy does prevent or severely impair reversal of two-tone discrimination learning of the rabbit NM response, (Orr, W.B. and Berger, T.W., Neurosci. Abstr., 7, 1981) a finding which is consistent with previous work using other behavioral paradigms (see Hirsh, R., Behav. Biol., 12, 1974). The present study, therefore, examined the electrophysiological activity of antidromically identified hippocampal neurons during two-tone discrimination and reversal learning of the rabbit NM response.

Under halothane anesthesia, all animals were prepared for single unit recording with implantation of microdrives overlying the dorsal hippocampus and bipolar stimulation electrodes into the fornix. Following two weeks of recovery, animals were trained in a two-tone discrimination task using either a 1K or 10K HZ tone as the CS+, a 1K or 10K HZ tone as the CS- (counterbalanced) and a corneal airpuff as the UCS. Single unit electrodes were lowered into the CA3 region of the hippocampus for extracellular recording of isolated neuronal activity during behavioral conditioning. A cell was identified as a pyramidal neuron if: i) a spontaneously occurring action potential could be successfully collided using fornix shock or ii) the spontaneous firing characteristics of the neuron matched those of cells that were collided. Results showed that during asymptotic discrimination performance, hippocampal pyramidal neurons exhibit a significantly higher frequency of discharge to the CS+ than to the CS-. In addition, as described in previous studies (Berger, T.W. et al., Brain Res., 193, 1980), the distribution of action potentials within conditioning trials correlates highly with the topography of the behavioral response.

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42.5 A NEURAL LOCUS WHERE A CONDITIONED STIMULUS ALTERS BEHAVIOR (ACOUSTIC STARTLE): SELECTIVE EFFECTS OF STIMULATION SITE AND DIAZEPAM. W.K. Berg* and M. Davis, Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508. SPON: M. SHEARD.

When a startle response is elicited in the presence of a stimulus previously associated with an aversive shock, startle magnitude will be increased. To determine the neural locus at which a conditioned stimulus alters this behavior, "startle" responses were elicited by electrical stimulation at specific points along the acoustic startle circuit to assess the extent of the circuit needed to mediate the conditioned effect.

Separate groups of male albino rats were implanted with chronic, bilateral electrodes in the ventral cochlear nucleus (VCN, n= 13), the nuclei of the lateral lemniscus (LL, n=8) or the nucleus reticularis pontis caudalis (RPC, n = 10). Following recovery animals received two daily training sessions with 10 light shock pairings in each session (1.1 sec light, 0.5 sec, 0.5 mA shock, 0.6 light-shock interval). During testing (1 to 3 days later) startle was elicited with either an acoustic (50 msec white noise burst) or an electrical (single, 1 msec cathodal pulse - 12-100 uA) "startle" stimulus. For half of each of the trial types, a light preceded the startle stimulus by 0.6 sec.

Consistent with previous studies in unoperated animals, the light increased acoustically elicited startle by 109%. The light also increased startle when elicited electrically in the VCN by 137% ($t(12) = 5.5$, $p < .01$). Moreover, within rats, there was a high correlation between the degree of potentiation of acoustic and VCN-elicited startle ($r(11) = .84$, $p < .01$). In contrast, potentiation did not occur when the response was elicited in the LL or RPC, suggesting that the conditioned stimulus modulates transmission at the VCN or LL, but not in the RPC or spinal cord. To distinguish between the former two sites, 7 rats were implanted with electrodes near the ventral acoustic stria, the fibers that connect the VCN to the LL. Each of these rats showed potentiated startle indicating that the sites beyond the VCN (e.g., the LL) must be involved. Diazepam (.625, 1.25 or 2.5 mg/kg) selectively decreased potentiation of VCN-elicited startle in the presence of the light in a dose-related manner without altering baseline startle amplitudes.

These results strongly suggest that the light modulates startle at the LL and demonstrate, perhaps for the first time in the vertebrate, a specific neural site at which a learned stimulus association alters a behavioral response. The LL may also be the site where diazepam acts to attenuate fear since this nucleus has one of the highest densities of benzodiazepine receptors in brain.

42.6 ORIENTING REACTION HABITUATION IN PATIENTS WITH EPILEPTOGENIC CEREBRAL TUMORS. R. Rogozea and V. Florea-Ciocoiu+. Inst. Neurol. Psychiat., Bucharest, ROMANIA.

An electrographic study on the resistance to habituation of the somatic (EMG), autonomic (finger plethismogram, galvanic skin reaction, respiration, pulse) and EEG (acoustic evoked potential, EEG-blocking reaction) components of the orienting reaction elicited by a repetitive auditory stimulus was performed in 41 patients with epileptogenic cerebral tumors and in 128 matched subjects in three control groups.

The study evidenced a significant higher resistance to habituation of the orienting reaction components in patients with epileptogenic cerebral tumors than in control subjects. Thus, in these patients, the somatic autonomic and EEG components of the orienting reaction necessiteted a significantly larger number of repetitive applications of auditory stimulus to become habituated.

The degree of habituation disturbances depended on the seizure frequency and preoperative history, on the features of interictal EEG tracings as well as on the tumor surgical removal. The most important increases in the resistance to habituation of the orienting reaction components were found in patients with frequent (daily) seizures, in cases with an over three-year preoperative seizure history, in patients with fast EEG rhythms or diffuse or focal irritative-type EEG abnormalities as well as in those with a shorter time elapsed from the tumor surgical removal.

The above-mentioned habituation disturbances of the orienting reaction components noted in patients with epileptogenic cerebral tumors should be related to the effects induced by the pathologic impulses arising from the nervous tissue surrounding the cerebral tumor on the neural structures and pathways involved in the regulation of nervous excitability and, implicitly, in the control and integration of repetitive sensory messages.

42.7 SEPARATE NEURAL MEDIATION OF DIFFERENT COMPONENTS OF ATTENTION. MaryLou Cheal. Neuropsychology Laboratory, Ralph Lowell Laboratories, Mailman Research Center, McLean Hospital and Harvard Medical School, Belmont, MA 02178.

Attention is not a unitary process either as expressed behaviorally or as mediated neurally. Behaviorally, different components of attention can be demonstrated, such as awareness of a stimulus, selective attention necessary for memory of a stimulus, maintenance of attention to a stimulus, and the shift of attention from one stimulus to another. Additionally, separate neural mediation of these different components of attention is inferred from psychopharmacolgical data. Previously, it was reported that scopolamine, the acetylcholine receptor blocker, disrupted maintenance of attention in gerbils (Neuroscience Abstracts, 1981, 7, 524). On the other hand, apomorphine, the dopamine (DA) receptor agonist, disrupted selective attention (Neuroscience Abstracts, 1979, 5, 644). In fact, even drugs with very similar neurochemical functions have different effects on different components of attention, and the same drug at different doses may have qualitatively different behavioral effects. Four different DA agonists have now been studied: amphetamine, apomorphine, L-DOPA, and piribedil (ET 495). Piribedil (10-300 mg/kg), like amphetamine, did not affect selective attention, whereas, L-DOPA (100 mg/kg) acted like apomorphine in disrupting selective attention. During the peak period of drug activity following this same dose of L-DOPA, the gerbils were able to respond to the stimulus and appeared to be aware of it. In distinction to the high dose effect of L-DOPA, a low dose of L-DOPA (10 mg/kg) or piribedil (30 mg/kg) caused a perseveration of responding to the stimulus, suggesting an inability to shift attention normally. Differential effects of these DA agonists on norepinephrine mechanisms may explain the difference in behavioral effects. An interaction of the NE receptor agonist, clonidine , and apomorphine was shown previously in these behaviors (Neuroscience Abstracts, 1980, 6, 813).

42.8 MEMORY RETENTION: EFFECT OF PROLONGED CHOLINERGIC STIMULATION IN MICE. James F. Flood*, Gary E. Smith* and Arthur Cherkin. GRECC, VA Medical Center, Sepulveda, CA 91343.

Many studies report improved memory retention following administration of acetylcholine precursors or of drugs which activate cholinergic receptors. A basic assumption is that an increased level or duration of cholinergic receptor activity will improve memory processing. We tested the duration hypothesis by prolonging the action of a cholinergic agonist, arecoline hydrobromide (ARE), by making two or three successive intraventricular injections, the first within 3 min after training, the second 90 min later, and the third after another 90 min. Memory, tested one week after training, was scored as the percent of mice (N=20/group) which met the criteria for memory retention in our T-maze active avoidance paradigm (Brain Res. 215, 77-185, 1981). Each injection of ARE was fixed at 50 ng/mouse, a dose which did not improve retention significantly when injected once (see Table, Groups 1-2).

The retention score for one ARE injection (35%) or two ARE injections (45%) did not differ significantly from the score for saline controls (25%), whereas three ARE injections caused marked improvement (95%; $p<0.01$, Dunnett's t-test). Delaying a single injection for 183 min after training gave a score of 30% for a dose of 50ng, and a score of 20% for a dose of 150ng, indicating that the effect of three spaced injections of 50ng each was not due simply to greater effectiveness at the 183-min delay, nor to the cumulative ARE dose (150ng) resulting from 3 injections. Also, three spaced saline injections gave a score of 25%, ruling out a non-specific enhancing effect of repeated injections. Our results support the hypothesis that prolonging the duration of cholinergic stimulation improves memory processing.

	INJECTION AT STATED INTERVAL POST-TRAINING (min)			RETENTION SCORE	
GROUP	3	93	183	(%)	REMARKS
1	ARE*	-	-	35	No sig. diff. from Gp. 2.
2	SAL	-	-	25	
3	ARE	SAL	SAL	20	Result of Gp. 6 is not due to multiple injections per se.
4	ARE	ARE	-	45	No sig. diff. from Gp. 2 or 9.
5	ARE	ARE	SAL	35	
6	ARE	ARE	ARE	95	Differs from Gp. 9 ($p<0.01$).
7	SAL	SAL	ARE	30	183-min interval is not critical for result of Gp. 6.
8	SAL	SAL	3xARE*	20	183-min interval is not critical for result of Gp. 6.
9	SAL	SAL	SAL	25	No diff. from Gp. 2.

*Each ARE injection is 50ng/mouse. The total Gp. 8 dose is 150ng.

42.9 FACILITATION OF RETENTION FOR PASSIVE AVOIDANCE CONDITIONING PRODUCED BY OPIATE ANTAGONISTS. Michela Gallagher. Dept. of Psychology, Univ. of North Carolina, Chapel Hill, NC 27514.

A number of studies, including our own, have reported that administration of naloxone, an opiate antagonist, facilitates time-dependent memory processes. This finding may indicate that naloxone enhances memory processes by blocking endogenous activation of opiate receptors. In support of this interpretation several of these previous studies have also reported that post-training opiate agonist administration impairs memory processes, an effect opposite to that produced by naloxone. However, naloxone possesses pharmacological activity which is not mediated by its opiate antagonist properties (Dingledine et al. Europ. J. Pharmacol. 47: 19, 1978; Baldino and Beckman. Brain Res. 232: 247, 1982). Therefore, the present study was undertaken to determine whether the effect of naloxone on memory processes is 1)shared by other opiate antagonists, and 2)exhibits stereospecificity.

Sprague-Dawley rats received one-trial step-through passive avoidance conditioning using the procedure described by Gallagher et al. (Science. 198: 423, 1977). Intraperitoneal injections were administered immediately after the training trial, and retention testing was conducted 24 hrs later. In addition to an uninjected control group and a vehicle-injected control group, opiate antagonist-injected groups received either naloxone (0.5, 1.0, or 2.0 mg/kg), diprenorphine (0.25, 0.5 or 1.0 mg/kg), or levallorphan (2.5, 5.0 or 10.0 mg/kg). A group receiving post-training administration of dextrallorphan (10.0 mg/kg), the inactive enantiomer of levallorphan, was also included.

Statistical analysis of retention test step-through latencies revealed that each of the three opiate antagonists significantly increased retention when compared to the control groups. For each opiate antagonist, the two higher doses were effective while the lowest dose did not significantly alter retention. In addition, the increased retention produced by levallorphan exhibited stereospecificity. Whereas levallorphan increased retention, a comparable dose of dextrallorphan did not produce a significant effect.

This experiment provides strong support for the interpretation that naloxone facilitates retention of passive avoidance conditioning as a function of its opiate antagonist properties. This effect of naloxone was shared by other opiate antagonists and was not observed following administration of the inactive enantiomer of one of these antagonists, levallorphan. These results suggest that memory processes may be, at least in part, controlled by endogenous opioid peptides which regulate opiate receptor activity.

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42.10 NALOXONE-INDUCED FACILITATION OF LATENT INHIBITION IN RABBITS. Elizabeth Bostock and Michela Gallagher. Neurobiology Program and Dept. of Psych., Univ. of N. Carolina, Chapel Hill, NC 27514.

A number of investigations have reported that administration of the opiate antagonist naloxone produces facilitation of time-dependent memory processes. Although the majority of these studies have used avoidance conditioning procedures, the effect of naloxone on memory processes does not appear to be restricted to tasks which employ aversive stimuli. In addition to retention of passive and active avoidance conditioning, naloxone has been observed to facilitate retention of habituation in rats. The present investigation was undertaken to investigate further the effects of post-training naloxone administration on retention of exposure to a non-aversive stimulus by using a latent inhibition procedure.

New Zealand albino rabbits in the latent inhibition groups received 15 tone (1 KHz, 82 dB, 5 sec) presentations per day for 3 consecutive days. On Day 4 all rabbits received a standard Pavlovian heart rate conditioning session in which 15 tone alone presentations were followed by 40 trials in which the tone was paired with an unconditioned stimulus consisting of eyelid shock (1.2 mA, 500 msec). The latent inhibition effect of tone pre-exposure is normally a decrement in subsequent conditioning. A normal conditioning group (Day 4 only), an uninjected latent inhibition group (Days 1-4), and three latent inhibition groups which received intraperitoneal injections on Days 1-3 immediately following the 15 tone presentations were included. These latter groups included a vehicle-injected group, a group receiving a 1.0 mg/kg injection of naloxone, and a group receiving two 0.5 mg/kg injections of naloxone spaced 30 min apart.

Statistical analyses revealed that compared to the normal conditioning group, the uninjected latent inhibition group exhibited a significant decrement in conditioned heart rate responding. A comparison of the uninjected and vehicle-injected latent inhibition groups revealed no significant differences in conditioned heart rate responding between these control groups. However, compared to the latent inhibition control groups, both of the groups which received naloxone administration exhibited a significantly greater latent inhibition effect.

The facilitation of latent inhibition produced by naloxone administration following the pre-exposure sessions is consistent with other reports that naloxone enhances time-dependent memory processes in rats. The present investigation appears to indicate that this finding generalizes to another laboratory species, the rabbit. Furthermore, these results provide additional evidence that the effect of naloxone on memory processes is not limited to retention of aversive experiences.

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42.11 OPPOSITE EFFECTS OF LEU-ENKEPHALIN AND METHYLNALOXONE ON AVOIDANCE CONDITIONING: POSSIBLE INVOLVEMENT OF PERIPHERAL OPIOID RECEPTORS. J.L. Martinez, Jr., K. Olson*, C. Hilsten*, and J. de Graaf*. Psychobiology Department, School of Biological Sciences, University of California, Irvine, CA, USA, and CNS Pharmacology Department, Organon, Oss, The Netherlands.

Previously, Martinez and Rigter (Neurosci. Abs., 1980, 6, 319) reported that the impairing actions of Met- and Leu-enkephalin on acquisition of a one-way active avoidance response in rats were altered by removal of the adrenal medulla. Adrenal medullectomy abolished the actions of Met-enkephalin and shifted the effective dose of Leu-enkephalin to higher doses, suggesting that enkephalin effects on conditioning may be related to peripheral mechanisms.

The present study was undertaken to determine whether methylnaloxone, an opioid antagonist, which has a limited ability to cross the blood brain barrier, would affect avoidance conditioning in a manner similar to naloxone, which crosses freely into the brain. Further, it was expected that the naloxones would affect conditioning in a manner opposite to Leu-enkephalin, an opioid agonist. Male Swiss-Webster mice (25-30 g) were trained in a discriminated Y-maze avoidance task. On trial 1 the door was opened, and the shock was turned on (1.0 mA). The shock was terminated when the mouse reached either the right or left goal arm. Following a 30-sec intertrial interval, the mouse was replaced in the start box. On trial 2 and all remaining trials (up to a total of 8), the mouse had 10 sec to enter the safe arm before the shock came on, and was trained to the side opposite its initial choice. An error was recorded if on trials 3-8, the mouse first entered the wrong goal arm or re-entered the start box.

Five minutes before training, the mice were injected i.p. with either Leu-enkephalin (Sal, 100 or 200 µg/kg), naloxone (Sal, 1 or 10 mg/kg), or one of two forms of methylnaloxone (Sal, 1 or 10 mg/kg): (1) methylnaloxone HCl, (2) methylnaloxone HBr. It was found that Leu-enkephalin (100 µg/kg, $p<.01$) impaired acquisition of the response, as these animals made more errors than their saline control group. Naloxone (1 and 10 mg/kg, $p<.02$), methylnaloxone HCl (10.0 mg/kg, $p<.05$), and methylnaloxone HBr (1 mg/kg, $p<.05$) all facilitated acquisition, as these animals made fewer errors than their saline control groups.

It is likely that Leu-enkephalin and the naloxones affected conditioning by acting on opioid receptors, since they had opposite actions. Further, since the methylnaloxones do not readily cross into the brain, and they affect conditioning at comparable doses to naloxone, it is likely that their actions are mediated through peripheral opioid receptors.

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42.12 EFFECTS OF PERIPHERAL EPINEPHRINE ADMINISTRATION ON THE EXTINCTION OF LEARNED FEAR. M.A. Downen* and R.A. Jensen. Department of Psychology, Southern Illinois University, Carbondale, IL 62901.

Previous research indicates that peripheral administration of catecholamines modulates acquisition and retention of learned responses. Epinephrine (E) administration reliably results in the enhanced acquisition and retention of avoidance responses. Of particular interest is the possibility that the behavioral effects of E are due to peripheral autonomic arousal. Thus, we investigated the effects of peripheral E administration on the extinction of a learned fear.

Earlier findings from our laboratory indicated that E retards the extinction of a step-up avoidance response in young rats. With mature rats, differential effects were seen according to the duration of footshock received on training day. With long footshock, E facilitated extinction as compared to saline control animals. However, extinction was retarded in animals receiving E and a short duration footshock compared to saline controls.

In the present experiments, animals were trained to a criterion of two consecutive step-up active avoidance responses with a 1.25 mA footshock. Extinction was achieved by placing the animals in the training apparatus with no shock for 6 min on the day after avoidance training. On testing day, there was a difference in the latency-to-step-up measure between the animals that were injected with saline that received an extinction trial (S-E) and those that were in the saline-no-extinction (S-NE) group. The 0.5 and 1.0 mg/kg doses of E decreased latency to step onto the shelf compared to the S-E group. The total time spent on the shelf was low for the S-E animals as compared to the S-NE animals. Again, 0.5 and 1.0 mg/kg E impaired extinction. These results using an active avoidance task generally indicate that the increased peripheral arousal induced by E impaired the acquisition of extinction of fear.

To assess the generality of the effect of E on extinction, an inhibitory avoidance task with low intensity footshock was used. The difference in step-through latency between S-E and S-NE rats was large with S-E animals showing more extinction. In this task administration of 0.5 mg/kg E prior to the extinction trial significantly enhanced extinction measured on the test day. Thus, the findings with an inhibitory avoidance task with low intensity footshock indicate that the increased arousal induced by E administration enhances the acquisition of extinction.

These differential drug effects on extinction might be due to the differing amount of footshock administered on training day in these two tasks. The effects seen fit an inverted U-shaped function for arousal induced by either shock intensity or dose of E and indicate that the degree of peripheral arousal modulates the degree of extinction of a learned fear.

43.1 TIME VARYING PULSATILE SEQUENCES FOR ELECTRICAL STIMULATION OF THE AUDITORY NERVE DERIVED FROM THE NEURAL ACTIVITY RECORDED IN RESPONSE TO COMPLEX SOUNDS. K. M. Mudry. Dept. of Electrical Engineering, Univ. of Akron, Akron, OH 44325.

VIIIth nerve auditory activity was examined in bullfrogs (*Rana catesbeiana*) to determine whether there are several basic sequences of action potential patterns occurring in response to simple and complex sounds.

Responses to single tones and combinations of two and three tones with various rise-fall times and intensities were analyzed. The combination tones were either phase-locked and phase-triggered or of random phase and unlocked frequencies.

The time of occurrence of the action potentials for each stimulus was stored in a mini-computer. For various driving signals, spike patterns were compared for fibers with similar and different best frequencies, thresholds and spontaneous activity levels. These comparisons between spike patterns were made through the use of post-stimulus, inter-spike-interval, and difference histograms, dot displays, power spectral analyses, and cross-correlation analyses.

To convey maximum information to the central auditory system through electrical stimulation of the VIIIth nerve with one electrode, a single pulsatile sequence must be used. Several suggestions for an appropriate pulsatile stimulation pattern will be presented. An ensemble of time varying pulsatile sequences chosen to maximize the information sent to the central auditory system via a two or three channel stimulation system implanted to drive certain populations of auditory fibers in the VIIIth nerve [either populations from the amphibian papilla and basilar papilla or populations tuned to low (75<BF<400 Hz), mid (400<BF<1000 Hz) and high (1000<BF<1800 Hz) frequencies] will be suggested.

Based on these studies the benefits of increasing the number of channels in an auditory prosthesis will be examined and the consequences of misplacement of electrodes on the information transmitted to the central nervous system will be discussed.

43.2 ORIGIN OF EFFERENT ACOUSTIC AND EFFERENT VESTIBULAR NEURONS IN VERTEBRATES. A COMPARATIVE STUDY. J. Strutz* (SPON: B. Fischer).Dept. Oto-Rhino-Laryngol. Unit f. Morph. Brain Res., Univ. Freiburg, West-Germany

Representatives of all classes of vertebrates (goldfish, tree frog, turtle, caiman, chicken, guinea pig) were investigated to determine the origin of centrifugal fibers to the inner ear. The anesthetized specimen received injections of a 50% aqueous solution of HRP (volume: 0.2 ul) either into the cochlear duct (to determine efferent acoustic neurons, exception:fish) or into the ampulla of the horizontal semicircular canal (to determine efferent vestibular neurons).For histochemistry we used Mesulam's TMB technique.

In the goldfish (Carassius auratus) labeled efferent labyrinthine neurons are restricted to the ipsilateral nucleus motorius tegmenti of the brain stem reticular formation, close to the midline and just lateral to the fasciculus longitudinalis medialis. In amphibians (frog, Hyla cinerea) parent cells are found more laterally in the ipsilateral reticular formation (Nucleus reticularis medius,Rm). In a primitive reptile (turtle, Terrapene ornata) efferent acoustic and efferent vestibular neurons appear bilaterally in the Rm, with a dorso-ventral extension from the abducens nucleus to the superior olive (OS). In contrast,in a highly developed reptile (Caiman crocodilus),efferent vestibular neurons remain bilaterally in the Rm, while efferent acoustic neurons form a separate population close to the OS. This resembles highly the situation found in avians (Gallus domesticus). The process of the spatial separation of the acoustic from the vestibular efferent cells is even more pronounced in mammals (guinea pig) where efferent vestibular neurons appear bilaterally in the phylogenetically old position in the Rm, and more dorsal in a position lateral to the genu of the facial nerve. The efferent acoustic neurons shift more ventrally and are found bilaterally in the superior olivary complex. In addition, neurons of the guinea pig's ventral nucleus of the lateral lemniscus participate in the efferent cochlear innervation.

In all species investigated, the primary vestibular and acoustic nuclei, respectively, are labeled by anterograde HRP transport. However, no labeled efferent cells are found in these nuclei.

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43.3 COLLATERALS OF LABYRINTHINE EFFERENT AXONS. J.C. Adams. Dept. of Otolaryngology and Anatomy, Medical University of S.C., Charleston, SC 29425.

Retrograde marking techniques were used to demonstrate that axons projecting to the labyrinth of the cat send collaterals to at least one other target. Following filling of the cochlea on one side with HRP, the labelled efferent bundle can be traced into the eighth nerve on the contralateral side. In such experiments a few labelled axons have been seen entering the contralateral cochlear nucleus. Application of fluorescent retrograde markers reveals patterns of the collateral innervation of the efferent systems. Application of retrograde markers in both cochleas results in double labelled vestibular efferent cells as well as double labelled large and small olivocochlear cells. Double labelled olivocochlear cells are also seen when one label is applied to a cochlea and another label injected into the ipsilateral or contralateral ventral cochlear nucleus. Double labelled cells were not seen when the second label was placed into either dorsal cochlear nucleus. These findings were supplemented by acetylcholinesterase staining in young animals. Labyrinthine efferent cell bodies and their axons stain darkly in the newborn and collaterals can be seen entering the ventral cochlear nucleus. Esterase staining in the superficial granular layer of the ventral nucleus and in the molecular/granular region of the dorsal nucleus is conspicuous by its absence in young animals. These results support the work of others who have evidence showing that esterase activity in superficial granule cell regions of the cochlear nucleus may not be due to terminals of olivocochlear collaterals. If vestibular efferent fibers have central collaterals the esterase staining indicates that they are in the interstitial nucleus of the vestibular nerve.

Supported in part by NSF grant BNS 81-10222 and by a South Carolina Biomedical Research Grant.

43.4 INTRACELLULAR RESPONSES OF CELLS IN MOUSE COCHLEAR NUCLEUS TO ELECTRICAL STIMULATION OF THE AUDITORY NERVE IN VITRO. D. Oertel. Dept of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706.

Intracellular recordings have been made from brain slices of cochlear nuclei of mice 2 to 4 weeks old. The slices consisted of the most lateral parasagittal section of the cochlear nucleus, 250 μm thick. They contained the stump of the auditory nerve, a large part of the anteroventral and parts of the posteroventral and dorsal cochlear nucleus. They were continually superfused with saline whose composition was changed during intracellular recordings. Cells were impaled under visual control and responses were recorded to electrical stimulation of the auditory nerve. Responses of 7 cells fall into 2 categories.

Two cells, about 0.5 mm anterior to the point of entry of the auditory nerve into the cochlear nucleus, responded to nerve stimulation with large (30 mV), brief (2 msec) depolarizations. The depolarizations were graded with stimulus strength and they were abolished reversibly when Mg^{2+} was substituted for Ca^{2+} in the saline. These cells had small (20 mV) action potentials whose shapes were not altered by the removal of extracellular Ca^{2+}.

Four cells, lying anterior or just dorsal to the point of entry of the auditory nerve, responded to nerve stimulation with an excitatory postsynaptic potential (about 10 mV, 5 msec) followed by a longer inhibitory postsynaptic potential (reversed at resting potential, -60 mV, 10 msec). A fifth cell responded with only an inhibitory postsynaptic potential. These cells had large (>40 mV) action potentials. One cell had action potentials which were not affected by the removal of extracellular Ca^{2+} while two others had action potentials whose undershoots were Ca^{2+} dependent.

None of 9 excitatory responses to auditory nerve stimulation recorded intra- and extracellularly, which were abolished by the removal of extracellular Ca^{2+}, was blocked by 5 x 10^{-5}M D-∝-aminoadipate (Martin, M.R. and Adams, J.C., Neuroscience 4:1097, 1979). None of 5 cells with Ca^{2+} dependent responses to auditory nerve stimulation was sensitive to 10^{-4}M l-glutamate superfused in the bath; none of 5 cells was sensitive to 10^{-4}M l-aspartate (Wenthold, R.J., Brain Res. 143:544, 1978).

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43.5 A PATHWAY CONNECTING THE RIGHT AND LEFT COCHLEAR NUCLEI IN THE CAT. Nell B. Cant and Karen C. Gaston*. Department of Anatomy, Duke University Medical Center, Durham, NC 27710.

Connections between the right and left cochlear nuclear complexes were studied with retrograde and anterograde axonal transport techniques. Very large multipolar neurons in the anterior and posterior divisions of the anteroventral cochlear nucleus and in the posteroventral cochlear nucleus project to the ventral and dorsal cochlear nuclei on the opposite side. In addition, giant cells in the deep layers of the dorsal cochlear nucleus project to the contralateral posteroventral cochlear nucleus and possibly also to the contralateral dorsal cochlear nucleus. The pattern of terminal distribution of the crossed connections was determined using anterograde axonal transport of horseradish peroxidase-labelled wheat germ lectin. Although no part of the cochlear nuclear complex is completely free of anterograde label, the densest labelling is found in the anterior division of the anteroventral cochlear nucleus, throughout the posteroventral cochlear nucleus, where it is closely associated with cell bodies, and in the fusiform and superficial layers of the dorsal cochlear nucleus.

These direct synaptic connections from one cochlear nucleus to the other might play a role in processes such as sound localization that depend on binaural interactions within the central nervous system.

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43.6 THE NUCLEI OF THE OWL'S AUDITORY BRAINSTEM ARE CHARACTERIZED BY UNIQUE MODES OF BINAURAL INTERACTION. A. Moiseff and M. Konishi. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

A neurophysiological correlate to sound localization has been described in the midbrain auditory nucleus of the barn owl, Tyto alba (Knudsen and Konishi, Science 200:795-797, 1978). Owls determine the location of a sound source from cues derived from ongoing-time disparities ("OTD", which in the case of tonal stimuli is equivalent to interaural-phase disparity) and interaural intensity differences ("ID") (Knudsen and Konishi, J. Comp. Physiol., 133: 1-11, 1979; Moiseff and Konishi, J. Neurosci., 1: 40-48, 1981). In preparation for an in-depth investigation of the neural mechanisms responsible for extracting the location of a sound source from these binaural cues, we have been characterizing the binaural interactions present in the nuclei of the auditory brainstem and midbrain.

The nuclei studied thus far are: olivaris superior (Os), the nuclei of the lateral lemniscus (Lemnisci lateralis, pars ventralis = LLv; ventralis lemnisci lateralis = VLV) and mesencephalicus lateralis, pars dorsalis (MLD, the avian homologue of the mammalian inferior colliculus). Units within these nuclei were tested for their monaural response to stimulation of the contralateral and ipsilateral ears as well as their response to the binaural parameters of OTD and ID. The responses to monaural stimuli were classified according to the unit's response relative to its spontaneous activity (excitation, inhibition, or no effect). Sensitivity to binaural stimuli were classified as "+" if the unit was sensitive or "-" if it was not.

The following table summarizes the responses found within the binaural areas of the specified nuclei to monaural and binaural stimulation:

	MONAURAL		BINAURAL	
NUCLEUS	CONTRALATERAL	IPSILATERAL	OTD	ID
Os	Excitation	Excitation	-	-
VLVp	Excitation	Excitation	+	-
VLVa	Excitation	Inhibition	-	-
LLv	Excitation	Inhibition	+	-
MLD*	Inhibition	Inhibition	+	+

(*area within MLD with restricted auditory receptive fields.)

Our results suggest that these nuclei are organized as functionally homogeneous groups of cells and can be classified according to their "physiological signatures." (Supported by a Helen Hay Whitney post-doctoral fellowship to A. M., and an NIH grant to M. K.)

43.7 FREQUENCY SELECTIVITY IN THE ROSTRAL MIDBRAIN/POSTERIOR THALAMUS OF THE LEOPARD FROG (RANA P. PIPIENS). Z.M. Fuzessery and A.S. Feng, Dept. of Biophysics and Physiol., Univ. of Illinois, Urbana, IL 61801.

A single unit study of frequency selectivity of auditory neurons in the anterior midbrain and posterior thalamus indicates that large percentage of these cells are either facilitated (threshold is decreased) by appropriate multiple tone stimuli, or require multiple tones for excitation. Such neurons were recorded in the rostrolateral torus semicircularis, tectum, pretectal gray, and the posterolateral and posterocentral thalamic nuclei. As a class, these neurons appear to be selective for low (100-600 Hz) and high (1200-2500 Hz) frequencies in the species receptor response range. Facilitated neurons responded to single low frequency tones, but typically only at high intensities (>90 dB SPL). This response threshold could be dropped as much as 40 dB if a single high frequency tone was added to the stimulus. Mid frequencies were either inhibitory, non-excitatory or excitatory only at low intensities (< 70 dB SPL) and becoming inhibitory at higher intensities. Neurons responding only to multiple tones were also selective for combinations of low and high frequency tones, and were either inhibited or not excited by mid frequencies.

Other neurons responded well to single tones, and were typically broadly tuned. In some cases, they displayed distinct bimodal or trimodal tuning curves. Some bimodally tuned neurons had two discrete excitatory areas, with best frequencies in the low and high frequency ranges. Single tone responders were often inhibited by mid frequencies at higher intensities, and in this respect were similar to the multiple tone responders.

The trend which emerges from this study is a gradation in selectivity for low and high frequency tones. At one extreme, neurons are broadly tuned to all frequencies, progressing to neurons responding to low or high frequencies, and finally to neurons which respond only to low and high frequencies.

The mating call of this species has a bimodal distribution of spectral energy, with energy peaks in the low and high frequency ranges. Therefore, some of these neurons have a frequency selectivity which appears to be suspiciously well tuned for the selective detection of this call.

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43.8 PERIODICITY ANALYSIS IN THE TIME DOMAIN BY COUPLED NEURONAL OSCILLATIONS IN THE AUDITORY MIDBRAIN OF THE GUINEA FOWL. G. Langner*(SPON: H. Scheich). Zool. Inst. der TH-Darmstadt, Schnittspahnstr. 3, 61 Darmstadt, BRD.

Periodic amplitude fluctuations with periodicities below 2 kHz are typical for many acoustic communication signals, e.g. human vowels and voiced consonants. In consequence analysis of such periodicities which in this case are amplitude modulated sine waves (AM) should be highly important for auditory systems. In the auditory midbrain nucleus (MLD) of 7 awake Guinea fowls (Numida meleagris) 420 units were recorded. About 20% of the analyzed units showed preferences for various combinations of the modulation frequency f_m and the carrier frequency f_c of AM-signals. For a given response maximum the relationship of the period $\tau_m = 1/f_m$ and the period $\tau_c = 1/f_c$ may be described by an empirical equation, the periodicity equation:

$$m \cdot \tau_m + n \cdot \tau_c = l \cdot \tau_1,$$

where m, n and l are little integers typical for a unit. τ_1 is a time constant of 0.4 ms, possibly the delay of a synaptic transmission in the auditory system. The temporal patterns of the neuronal responses support this interpretation. Amplitude fluctuations like stimulus onsets or the waves of the envelopes of the AM-signals elicit spike trains oscillating with periods which are multiples of $\tau_1 = 0.4$ ms. These oscillations may be coupled strongly to a certain f_m as a function of f_c. Variation of f_m or f_c shifts the mean delay of the phase coupled activity proportional to $m \cdot \tau_m$ or $n \cdot \tau_c$, respectively. These effects are explained with input activity phase coupled to f_c which coincides at the level of the recorded units with oscillating input coupled to f_m. The coincidence condition is described by the periodicity equation given above.

Psychophysical pitch matching experiments were performed by presenting AM-signals to 5 human subjects. The pitch was measured by matching it to the pitch $P = 1/\tau_p$ of a pure tone. The results indicate that the postulated mechanisms and the periodicity equation are adequate for the explanation of periodicity pitch in humans. Hence the period τ_p of the perceived pitch may be described by the equation:

$$\tau_p = n \cdot \tau_c - l \cdot \tau_1,$$

where n and l are integers and $\tau_1 = 0.4$ ms. Furthermore plots of τ_p as a function of τ_c reveal steps at 0.4 ms intervals indicating that the neuronal time constant is the same in the bird and the human (Langner, G., Exp.Brain Res., 44:450-454,1981).

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43.9 ORGANIZATION OF MEDIAL SUPERIOR OLIVARY PROJECTIONS TO THE INFERIOR COLLICULUS IN THE CAT: REGIONAL SEGREGATION WITHIN THE CENTRAL NUCLEUS. Craig K. Henkel and Kevin M. Spangler*, Department of Anatomy, Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103.

Recent studies based on retrograde transport of HRP suggest that the projection of the medial superior olivary (MSO) nucleus to the inferior colliculus is both tonotopic and regionally segregated. In the present investigation autoradiographic experiments in the cat were correlated with HRP studies in order to reexamine the scheme of midbrain projections from MSO. In five cats HRP injections involved parts of the inferior colliculus. Throughout MSO on the side of large HRP injections, a large proportion of cells were labeled; in contrast, on the contralateral side, labeling was confined mainly to a few cells in the ventral pole of MSO. Consistently, laterally placed HRP injections in the central nucleus resulted in labeling of a larger number of neurons in MSO than in medially placed injections. Injection sites restricted to either the dorsomedial region or caudal section of the central nucleus labeled no MSO neurons. The distribution of MSO fibers in the midbrain was mapped in seven experiments with tritiated leucine injections in the superior olivary complex. Projections to the inferior colliculus in each case were distributed to the ventrolateral division of the central nucleus and always spared a section of the nucleus caudal to the commissure of the inferior colliculus. The most ventrally placed injections in MSO, corresponding in position to its highest frequency representation, labeled fibers ending within an obliquely oriented zone typical of the laminae described in the central nucleus of the inferior colliculus. Dorsomedially the labeled district approached a vertical mid-collicular line but did not appear to end within the dorsomedial division of the central nucleus. As isotopic injections involved more dorsal parts of MSO, labeled fibers ended in a more vertical array in the central nucleus and even more laterally. Autoradiographic results together with HRP experiments support previously described features of a scheme, though somewhat more restricted, for MSO projections to the inferior colliculus. Since the lateral projection field of MSO in the inferior colliculus appears to overlap with the projection of another binaurally responsive structure, the dorsal nucleus of the lateral lemniscus, it may reveal a pattern for functionally partitioning the ventrolateral subdivision.

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43.10 HORSERADISH PEROXIDASE DEMONSTRATION OF AFFERENT AND EFFERENT CONNECTIONS OF THE INFERIOR COLLICULUS C. J. Jones-Mumby*, C.O. Trouth, E. J. Moore*, J. A. Holloway, and M. Odek-Ogunde*, Laboratory of Neurophysiology, Howard University College of Medicine, Washington, D. C. 20059.

Following electrophysiological identification of units responding to acoustic clicks and/or electrical stimulation of the contralateral cochlear nucleus (CN), horseradish peroxidase (Sigma VI, 30-50% solution) was iontophoresed into the ventral, dorsal, and medial portions of the central nucleus of the inferior colliculus (ICC) of 20 cats. The results revealed:

I. Retrograde connections with: A. ventral ICC: Ipsilaterally-from medial (MSO) and lateral (LSO) superior olives; periolivary nuclei (PON); and in pairs of clusters from ventral nucleus of lateral lemniscus (VNLL). Contralaterally-from the fusiform cell layer of dorsal portion of dorsal cochlear nucleus (DCN); central postero-(PVCN) ventral and dorsal antero-(AVCN) ventral cochlear nuclei; nucleus paragigantocellularis lateralis (NPL); reticular division of substantia nigra (SNR). Bilaterally from the dorsal nucleus of lateral lemniscus (DNLL). B. dorsal ICC: Ispilaterally from LSO and MSO, VNLL (in pairs of clusters); and scattered throughout CN and PON; Contralaterally from the superficial to middle fusiform and molecular cell layers of the ventral DCN; ventral PVCN and AVCN. Bilaterally from DNLL. C. medial ICC: from Ipsilateral LSO and MSO.

II. Anterograde connections with ventral, dorsal, and medial ICC: Ipsilaterally - brachium (BIC) and nucleus (NBIC) of brachium of inferior colliculus; medial geniculate body (MGB). These anterograde findings were verified by the identification of retrograde labelling in the ICC following injections into corresponding regions of the dorsal, medial, and ventral MGB.

Following injections into the dorsal (low frequency region) and ventral (high frequency region) ICC, reaction-product was observed in the ventral (low frequency region) and dorsal (high frequency region) DCN respectively. These data confirm the existence of tonotopicity between ICC and CN nuclei, perhaps involving frequency specific cell forms. Further, the findings suggest that the output of the ICC is tonotopically organized. This is inferred from the identification of labelling in the lateral region of the ventral (low frequency region) MGB subsequent to injections in the dorsal ICC.

43.11 CRITICAL PERIOD FOR CENTRAL AUDITORY DEVELOPMENT IN CBA/J MICE. D. B. WEBSTER. Kresge Hearing Research Lab., Dept. of Otorhinolaryngology, LSU Med. Cntr., New Orleans, LA 70119.

In order to determine the time period when a conductive hearing loss affects the size of cochlear nucleus neurons, 9 groups (7 mice per group) of CBA/J mice had their left external auditory meatus removed at varied days after birth (DAB) and were sacrificed for anatomical analyses following different survival periods. All brains were serially sectioned, stained with cresyl violet, and the cross-sectional areas of 30 globular cells of the ventral cochlear nucleus were measured from both right and left side of each brain. Blind procedures were used to avoid observer bias. In mice operated on at 4 DAB and sacrificed at 12 DAB, there was no significant size difference between right and left sides ($p>.05$) and the neurons were of adult size. In mice operated on at 4 DAB and sacrificed at 18 DAB, the globular cells were 9% smaller on the left than on the right ($p<.01$). For those mice operated on at 4 DAB and sacrificed at 24, 45, and 90 DAB the globular cells were 21%, 16%, and 16% smaller on the left side than on the right (all $p<.01$). Left meatal removal at 12 DAB and sacrifice at 24 DAB resulted in neurons 19% smaller on the left ($p<.01$). Meatus removal at 12 DAB and sacrifice at 18 DAB resulted in neurons 14% smaller on the left ($p<.01$), while removal at 18 DAB and sacrifice at 24 DAB resulted in neurons 10% smaller on the left than on the right ($p<.01$). Left meatal removal at 45 DAB and sacrifice at 90 DAB resulted in only a 1% difference in globular cell size between sides ($p>.05$). In all the mice analyzed, the globular cells on the right (normal) side were of adult size. I am currently preparing mice whose left meatus is removed at 24 DAB and sacrificed at 45 DAB. Unilateral meatal removal causes an approximately 19% reduction of globular cell somata size between 12 and 24 DAB. Meatal removal does not affect globular cell cross-sectional area before 12 DAB and removal after 45 DAB also does not affect globular cell size. There is, therefore, a critical period in mouse development when a conductive loss causes a significant shrinkage of globular cell somata.

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44.1 SELECTIVE SPINAL CORD AMINO ACID ABNORMALITIES IN FRIEDREICH'S ATAXIA. Roger F. Butterworth and Jean-François Giguère*. Lab. of Neurochemistry, Clinical Research Inst. of Montreal (University of Montreal), 110 Pine Ave. w., Montreal Québec Canada H2W 1R7.

Spinal cord lesions in Friedreich's Ataxia invariably include loss of fibres in the gracile funiculi and in the pyramidal and dorsal spinocerebellar tracts. The present study was initiated to examine the possibility that loss of these specific neuronal tracts in Friedreich's Ataxia might be accompanied by changes in the distribution of certain cerebral amino acids suggested to be involved in spinal cord neurotransmission. Spinal cord samples from cervical, thoracic and lumbar levels, obtained at necropsy from four cases of Friedreich's Ataxia and ten controls were dissected into the following regions; spinal grey matter, dorsal columns, dorsal white matter and ventral white matter. The amino acids glutamate, glutamine, glycine and aspartate were measured in these regions by a sensitive double isotope dansyl microassay as previously described (Butterworth et al., J. Neurochem., 38, (1982). Glutamate and glutamine concentrations were found to be decreased by up to 70% in grey matter of Friedreich's lumbar cord. Glutamine levels were reduced to a similar extent in affected dorsal columns. No differences were observed at higher (cervical and thoracic) levels. These findings are consistent with a loss of nerve terminals and axons of affected primary sensory neurons and of descending corticospinal tracts, both of which have been suggested by previous experiments to utilise glutamate as neurotransmitter. A third group of fibres affected in Friedreich's Ataxia, namely those of the dorsal spinocerebellar tract originate in the Clarkes Nuclei and terminate in the cerebellar vermis as the putatively glutamatergic mossy fibres. Glutamate concentrations were recently found to be significantly reduced in cerebellar vermis of two Friedreich's patients (Huxtable et al., Can. J. Neurol. Sci., 6, 255, 1979). The findings from the present study when taken in conjunction with those of this earlier study afford evidence for selective degeneration of long, large diameter glutamatergic nerve fibres in Friedreich's Ataxia.

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44.2 DEPRESSED NOREPINEPHRINE UPTAKE BY SYNAPTOSOMES ISOLATED FROM HYPOTHALAMUS AND BRAIN STEM OF HYPERTENSIVE RATS. K. Hough*, J. Rho, B. Newman and N. Alexander (Spon: D.F. Lindsley). Depts. of Medicine and Anatomy, University of Southern California Schools of Medicine and Pharmacy, Los Angeles, CA 90033.

In order to study a possible alteration of the transmembrane norepinephrine (NE) uptake system in spontaneously hypertensive rats (SHR), we have studied the uptake processes directly in the synaptosomes isolated from the hypothalamus and brain stem of SHR. The synaptosome-enriched subfraction was prepared by flotation, employing our modification of a discontinuous Ficoll-sucrose gradient (Booth and Clark, Biochem. J. 176:365, 1978). The synaptosomes obtained by our procedure are morphologically relatively pure with more intact synaptosomes than previous preparation, and demonstrate that NE uptake is highly sodium-dependent (80%) and ouabain sensitive (70%). The initial ^{3}H-NE uptake by the hypothalamic synaptosomal fraction of SHR during the first 10 minute incubation period averages 1.065 ± 0.32 p moles per mg protein while that of Wistar Kyoto (WKY) control was 1.545 ± 0.36 p moles per mg protein. Thus, the ^{3}H-NE uptake by SHR samples in the initial 10 minute incubation period was considerably lower than that of WKY (31.1%) and the difference was statistically significant ($p<0.01$). Synaptosomes were also prepared from brain stem regions, in parallel with hypothalamic synaptosomes, from the same animals. In four comparative studies between SHR and WKY, in which two SHR stems and two WKY stems were combined per experiment, WKY also had greater uptake in each experiment averaging 36% higher uptake of NE per mg protein during the first 10 minute incubation period. These findings may together indicate an alteration of a membrane transport mechanism in genetic hypertension.

44.3 GLUTAMIC ACID BINDING IN FIBROBLASTS FROM PATIENTS WITH HUNTINGTON'S DISEASE. P.T.-H. Wong, V. K. Singh and E. G. McGeer. Kinsmen Laboratory of Neurological Research, University of British Columbia and Division of Immunology, Children's Hospital, Vancouver, B. C., Canada.

It was recently reported that fibroblasts from Huntington's disease (HD) patients exhibit hypersensitivity to glutamate toxicity and degenerate at a rapid rate when challenged with glutamic acid.[1] We therefore attempted to confirm these findings and to investigate whether the binding of glutamate to HD fibroblast membrane is significantly altered when compared to controls.

Fibroblasts from clinically unaffected subjects were found to be 88% viable in normal medium as determined by the trypan blue exclusion method. HD fibroblasts, however, were only 66% viable. This conflicted with the observations of Gray et al.[1] that cultures from both sources were more than 98% viable. They further reported that glutamate at 30 mM concentration was toxic to only HD fibroblasts, reducing cell viability to 6%. In contrast, we observed reductions of cell viability by glutamate to 39% and 8% with control and HD fibroblasts, respectively. In other words, glutamate decreased cell viability of control cultures by 55% (from 88% to 39% viability) but by 88% (from 66% to 8% viability) with HD cultures. Although differing in detail from those of Gray et al., the present results substantiate the conclusion that HD fibroblasts are more susceptible than control fibroblasts to glutamate toxicity.

Specific binding of [^{3}H]glutamate was obtained by measuring binding at concentrations ranging from 30-1000 nM in the presence and absence of 100 µM unlabelled glutamate. On linear regression analysis of the Scatchard plots, both HD and control fibroblast membranes showed single straight lines ($r = 0.99$) with K_D of 295 nM and B_{max} of 88 pmol/mg protein for HD fibroblasts and 462 nM and 175 pmol/mg protein, respectively, for control fibroblasts. Hill plots also showed straight lines ($r = 1$) with slopes of 1.

[1]Gray, P.N., May, P.C., Mundy, L. and Elkins, J., Biochem. Biophys. Res. Comm. 95:707-714, 1980.

Supported by the Huntington Society of Canada.

44.4 NEUROTENSIN SYSTEMS IN HUMAN SUBSTANTIA NIGRA: NORMAL/PARKINSON'S DISEASE. G. R. Uhl, P. J. Whitehouse*, C. L. White III*, J. C. Hedreen, R. E. Carraway*, D. L. Price*, and M. J. Kuhar. Departments of Neuroscience, Neurology, and Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205. Department of Biology, University of Massachusetts, Worcester, MA 01605.

Several lines of evidence indicate that neurotensin systems are present in the substantia nigra (SN) of the rat. Immunocytochemical studies show neurotensin fiber and terminal pathways of moderate density, and in vitro labeling with [^{3}H] neurotensin followed by autoradiography demonstrates dense neurotensin receptors in this region. Moreover, lesions produced by injection of 6-hydroxydopamine suggest that these receptors are associated with the dopaminergic neurons of the SN (Palacios, J.M. and Kuhar, M.J., Nature 294:587-589, 1981). Finally, when iontophoresed into the SN, neurotensin causes excitation.

With this as background, we examined the distribution of pre- and postsynaptic neurotensin markers in brains from human controls and a patient with Parkinson's disease (PD), a disorder associated with loss of pigmented dopaminergic neurons in the SN. Using rabbit antineurotensin serum and a conjugated peroxidase reagent, we have visualized immunoreactive fibers and terminals in low-to-moderate density in the pars compacta and reticulata of the SN. Fibers are not demonstrated when the antiserum is preincubated with neurotensin (10^{-5} M). Using in vitro labeling with [^{3}H] neurotensin, autoradiography discloses high densities of label in the SN and much lower grain densities over the adjacent cerebral peduncle. Grains cluster around pigmented perikarya of compact neurons. As predicted (Uhl G.R., Prange, N., and Nimeroff, A.J., N.Y. Acad. Sci., in press), the SN in PD shows a substantial loss of neurotensin receptors (to 30% of control values in preliminary quantitative studies).

The demonstration of neurotensin afferents in the SN and the finding that neurotensin receptors are expressed by pars compacta cells in the human SN suggest a role for this peptide in nonpyramidal motor control systems. In PD, loss of SN cells is associated with loss of receptors for this peptide, and changes in this system may have a role in the clinical expression of PD. Information concerning the anatomy and functional properties of this neurotensin system may have implications for therapy of PD and other movement disorders. (Supported by NIH MH 25951, MH 00053, NS 10580, and NS 15721.)

44.5 AMYOTROPHIC LATERAL SCLEROSIS: ALTERATIONS IN NEUROTRANSMITTER RECEPTORS. P.J. Whitehouse*, J.K. Wamsley, M.A. Zarbin*, D.L. Price, W.W. Tourtellotte*, L. Davis* and M.J. Kuhar (SPON: W.S. Young, III). Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Amyotrophic lateral sclerosis (ALS) is a degenerative neurological disease characterized by selective loss of upper and lower motor neurons. At autopsy, ALS spinal cords show loss of motor neurons, although it is now believed that the affected nerve cells do not die suddenly but exhibit a variety of structural/functional changes which antedate cell death. Expression of neurotransmitter receptors is one of the principle functional properties of nerve cells and might be expected to be altered early in the disease process. Knowledge of the receptor populations at risk in ALS would provide information concerning normal transmitter - specific circuitry as well as the consequences of diseases on these systems.

In the present study, muscarinic cholinergic, glycinergic, benzodiazepine, GABAergic and adrenergic receptors have been examined in normal and ALS tissue by an in vitro labeling autoradiographic method (Young and Kuhar, Brain Res. 179:255, 1979). Tissues from cervical, thoracic, and lumbar spinal cords, obtained from six patients with ALS and six controls (matched for age, sex and postmortem delay in autopsy) were labeled with [^{3}H] drugs by standard methods and autoradiographs prepared by using emulsion-coated coverslips or tritium-sensitive film (Palacios et al., Neurosci. Lett. 25:101, 1981).

In normal cords receptors were heavily concentrated in Rexed layers II-III and particularly with cholinergic and glycinergic in layer IX. The results are the first quantitative analysis of the distribution and density of these receptors in normal human spinal cord, and documents, at the light microscopic level, the severe loss of muscarinic cholinergic (particularly high affinity), benzodiazepine and glycine receptors in association with degeneration of motor neurons in ALS.

Loss of neurons appears to be the most obvious cause of loss of receptor binding in ALS. Other mechanisms such as alterations in affinity for ligand or blockade by exogenous or endogenous agents seem unlikely. Examination of animal models of ALS in which studies of the evolution of the disease are possible may provide further insight into disease mechanisms. Since receptors are neuronal markers, indicators of connectivity, and sites of drug action, the use of receptor mapping in neurological disorders may facilitate our understanding of pathophysiology and direct attempts at drug therapy.

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44.6 ALTERATIONS IN REGIONAL BRAIN LEVELS OF NEUROTENSIN, THYROTROPIN-RELEASING HORMONE AND SOMATOSTATIN IN SCHIZOPHRENIA AND HUNTINGTON'S CHOREA. C.B. Nemeroff, P.J. Manberg*, W.W. Youngblood*, A.J. Prange, Jr. and J.S. Kizer*, Biological Sciences Research Center, University of North Carolina School of Medicine, Chapel Hill, N.C. 27514

A variety of peptides have recently been isolated and characterized in brain tissue. Several of these may be neurotransmitters. The present study was designed to determine whether alterations in regional brain levels of three endogenous peptides, thyrotropin-releasing hormone (TRH), somatostatin (SRIF) and neurotensin (NT), occur in patients with schizophrenia or Huntington's chorea. Human brain tissue was collected and dissected at the MRC Brain Bank in Cambridge, England using previously described methods (Brain 102:333-346, 1979) and kindly provided by Dr. Martin Rossor. We examined brain samples from 50 patients without psychiatric or neurological disease (mean age 71 years, range 27-103 years), 46 schizophrenics (mean age 58 years, range 20-86 years) and 24 Huntington's choreics (mean age 54 years, range 26-78 years). The frozen brain samples were weighed, homogenized in 20 volumes (w/v) of 1N HCl, centrifuged at 12,000g for 30 min and the clear supernatants lyophilized. Radioimmunoassay procedures previously described were utilized to determine the regional brain concentrations of NT-like (J. Neurochem., in press), TRH-like (J. Neurosci. Meth. 4:305-314, 1981), and SRIF-like immunoreactivity (Endocrinol. 101:613-622, 1977). The concentrations of all three peptides were significantly increased in the caudate nucleus of the Huntington's patients, but not of the schizophrenic patients. No significant group-related alterations in the levels of any of the peptides studied were observed in the hypothalamus. However, there was a three-fold rise in SRIF-like immunoreactivity in the nucleus accumbens tissue of the Huntington's patients. In addition the Huntington's patients had significantly higher levels of TRH-like immunoreactivity in the amygdala. Brodmann's area (BA) 24 (cingulate cortex) showed no alterations in the concentration of any of the three peptides in samples from schizophrenic patients when compared to controls. However in schizophrenics, levels of TRH-like immunoreactivity were significantly decreased in two areas of frontal cortex (BA 12 and 32). Levels of SRIF-like immunoreactivity were significantly reduced in BA 12, and levels of NT-like immunoreactivity were significantly increased in BA 32. Whether these alterations in peptide levels in the CNS of patients with schizophrenia and Huntington's chorea are involved in the pathogenesis or behavioral aspects of these diseases is unknown.

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44.7 SELECTIVE SENSITIVITY OF DOPAMINE NERVE TERMINALS IN CEREBRAL ISCHEMIA - EVALUATION OF THE POTENTIAL PROTECTIVE EFFECT OF PARGYLINE. J. Weinberger* and G. Cohen. Dept. of Neurology, The Mount Sinai Sch. of Med., New York, N.Y. 10029

The study of energy-dependent, high affinity uptake of radiolabelled transmitters has been employed as a paradigm of neuronal membrane function in ischemia. Previous studies (Weinberger, J., Cohen, G., J. Neurochem., 38: 963, 1982) have shown a significantly greater reduction in dopamine uptake relative to glutamate after 16 hours of unilateral cerebral ischemia in the Mongolian gerbil. We investigated whether the monoamine oxidase (MAO) inhibitor pargyline could prevent the selective destruction of dopamine nerve terminals by preventing the formation of hydrogen peroxide and/or derived oxygen radicals.

Unilateral cerebral ischemia was induced in the Mongolian gerbil by left carotid ligation. Pargyline 75 mg/kg was administered i.p. 2 hours prior to ligation. 9/34 (26.5%) of pargyline treated animals and 8/32 (25.0%) of the untreated animals developed stroke. There were 2 deaths in each group. Seven treated and 8 untreated animals exhibited milder circling behavior.

Synaptosomes were prepared by differential centrifugation in 0.32M sucrose. Uptake was carried out in Krebs Ringer phosphate buffer pH 7.4, at concentrations of 5×10^{-9}M for dopamine and 8×10^{-6}M for glutamate. Synaptosomes were separated from the incubation medium by filtration through 0.65 micron Millipore filters for subsequent scintillation counting of ^{3}H-dopamine and ^{14}C-glutamate within the synaptosomes. The uptake into the ischemic (left) and control (right) hemispheres was expressed as the L/R ratio. The extent of reduction of uptake at 16 hours in animals with stroke is shown in the following table (mean ± SEM):

	Dopamine	Glutamate
Pargyline Treated	0.214 ± .043*	0.369 ± .033
Untreated	0.147 ± .024**	0.316 ± .012

The greater reduction in uptake of dopamine compared to glutamate was significant in treated and untreated animals (*$p < .02$, **$p < .001$).

Although uptake of dopamine was improved by 45% in pargyline treated animals, the data did not achieve statistical significance in this limited series. A greater protective effect may have been precluded if increased levels of endogenous vasoactive monoamines resulted in a more severe circulatory disturbance in the pargyline treated animals.

44.8 RECEPTOR BINDING CHANGES IN ZINC AND COPPER DEFICIENT RATS. J.D. Geiger*, J.C. Wallwork*, P.K. Seth*, L.M. Klevay*, H.H. Sandstead*, and S.S. Parmar. Departments of Physiology and Surgery, University of North Dakota School of Medicine and USDA, ARS, Human Nutrition Research Center, Grand Forks, ND 58202.

Trace metal nutriture has been shown to affect brain neurotransmitters which may lead to alterations in receptor binding and function. The effects of zinc and copper deficiency on receptor binding were investigated. Male Long-Evans weanling rats were divided into 3 groups for the zinc study; zinc deficient (ZD), pair fed (PF) and ad libitum-fed (ALZ). Zinc deficiency was produced by a standard method (Wallwork et al., Br. J. Nutr. 45: 127, 1981). Zinc deficiency was verified by a decrease in femur zinc levels by atomic absorption spectrometry. The zinc levels of 286 ± 24, 292 ± 39 and 93 ± 7 µg/g ($P < 0.0001$) were found in ALZ, PF and ZD rats, respectively. Weanling male Sprague-Dawley rats were rendered copper deficient using a standard method (Klevay, Am. J. Clin. Nutr. 26: 1060, 1973). Two groups of animals were used for the copper study; copper deficient (CD) and ad libitum-fed (ALC). Copper deficiency was verified by a decrease in hematocrit of 31% ($P < 0.001$). Brain regions from control and deficient animals were isolated and crude synaptic membranes were prepared for binding studies (Seth et al., Toxicol. Appl. Pharmacol. 59: 262, 1981).

In general, no significant changes between PF and ZD animals were observed in binding affinity for ^{3}H-diazepam (benzodiazepine), ^{3}H-muscimol (GABA), ^{3}H-spiroperidol (dopamine), ^{3}H-5HT (serotonin), and ^{3}H-QNB (muscarinic) receptors in brain preparations. However, significant changes were observed between ALZ and PF animals as evidenced by an increase in ^{3}H-QNB binding to cerebellar membranes from 17 ± 3 to 35 ± 7 pmol/g protein ($P < 0.01$). The K_D values for ^{3}H-spiroperidol binding to striatal membranes were 0.86 (ZD), 0.43 (PF) and 0.48 (ALZ). The B_{max} values for ZD, PF and ALZ rats were 757, 638 and 599 fmol/mg protein, respectively. The binding of ^{3}H-QNB to striatal and cerebellar membranes from ALC and CD rats increased from 595 ± 66 to 704 ± 82 ($P < 0.02$) and 19 ± 4 to 28 ± 7 ($P < 0.01$) pmol/g protein, respectively. ^{3}H-muscimol binding also increased in cerebellar membranes from 39 ± 12 to 53 ± 9 ($P < 0.02$) pmol/g protein. ^{3}H-Diazepam binding in brain preparations from CD rats decreased from 92 ± 18 to 75 ± 14 ($P < 0.02$) pmol/g protein.

Copper deficiency had no significant effect on ^{3}H-spiroperidol (striatum) and ^{3}H-5HT (frontal cortex) binding. Copper deficiency appears to affect receptor binding more profoundly than does zinc deficiency, although nonspecific starvation influences can not be discounted. These results may explain some of the manifestations of trace metal deficiency in animals. (Supported in part by the Dakota State Aerie Fraternal Order of Eagles)

44.9 DOPAMINERGIC REGULATION IN ANOREXIA NERVOSA. W.P. Owen, K.A. Halmi, E. Lesley* and P. Stokes., NY Hospital, White Plains, NY 10605.

Hypothalamic anatomical structures regulating feeding, sexual behavior and menstrual activity, all of which are disturbed in anorexia nervosa, are strongly influenced by dopaminergic activity. Lack of response of growth hormone (GH) to L-DOPA in weight restored anorectics indicate that dopaminergic regulation of GH secretion is impaired in anorexia nervosa. Since prolactin (PRL) secretion is also influenced by dopamine (DA), another indirect assessment of dopaminergic activity is the measurement of PRL response to a DA antagonist such as chlorpromazine (CPZ). Failure of anorectics to respond to clomiphene adequately and to establish a mid-cycle peak of leuteinizing hormone (LH) secretion is a phenomen that is seen in hyperprolactinemia. However, a survey of the literature reveals less than 10% of anorectic women have elevated basal prolactin levels. Could a central decrease in dopaminergic activity which should increase PRL secretion be masked by increased cortisol secretion which suppresses PRL? In order to access dopaminergic regulation of PRL secretion in anorexia nervosa, we measured PRL response to CPZ while concommitently assessing cortisol secretion.

Method - Six female recently treated normal weight anorexia nervosa patients had GH, PRL, and cortisol levels measured at AM fasting, 2 and 8 hours after 50 mg CPZ on testing days 1 and 2. At 11 PM on day 1 the patient received 1 mg dexamethasone (DX).

Results - Two of the six patients had a negligible PRL response to CPZ (21 ng/ml to 27 ng/ml and 14 ng/ml to 23 ng/ml). Another two patients had a minimal response (24 ng/ml to 35 ng/ml and 15 ng/ml to 27 ng/ml). Two patients had an adequate response (34 ng/ml to 62 ng/ml and 27 ng/ml to 100 ng/ml). Clearly dopaminergic regulation of PRL secretion is impaired in two patients and could be considered less than optimal in two other patients. Since anorectics have an impaired response of GH to L-DOPA, as well as an impaired response to a dopamine antagonist, one may hypothesize anorectics have a deficiency of the dopamine post-synaptic receptor sites. Two patients did not suppress to DX. One of these had a normal PRL response to CPZ and the other had a negligible response. Dopaminergic regulation of prolactin seems to be independent of the endogenous hypothalamic mechanisms which determine response to DX in anorexia nervosa. More patients need to be studied in order to access the relationship of resistance to DX to dopaminergic regulation of GH and PRL.

44.10 GLUTAMATE EXCITOTOXICITY IN LOCUST MUSCLE: A POTENTIAL MODEL SYSTEM FOR AMINO ACID CYTOTOXICITY IN VERTEBRATE CNS Ian R. Duce*, P. Lynn Donaldson+, and Peter N.R. Usherwood*. +Dept. of Physiology & Biophysics, Univ. of Iowa, Iowa City, Ia. 52242, and *Dept. of Zoology, Univ. of Nottingham, Nottingham, England (SPON: by R. Hahin).

Kainic acid (KA), an amino acid analogue of glutamate, injected into vertebrate central nervous systems causes selective degeneration of neurons in various loci of the CNS (e.g., the striatum, cerebellum, and hippocampus). One hypothesis for the selective neuronal degeneration in specific regions is that KA is excitotoxic to neurons with glutamate receptors. We examined this hypothesis in locust muscles, which have well characterized glutamate receptors. Locust retractor unguis muscles were dissected and isolated in small petri dishes. Some muscles were exposed to Concanavalin-A (1 uM) which blocks desensitization of glutamate receptors. Bath application of 1 mM L-glutamate caused degeneration of the muscle fibers which was first observed as an increased opacity in the muscle after 30 min. The ultrastructure of these muscles was marked by vacuolation of the terminal cisternae of the sarcoplasmic reticulum. Muscles not pretreated with Con-A but exposed to glutamate for the same time period did not degenerate. Longer exposures (up to six hours) produced more extensive damage. Analogues of glutamate were effective in initiating muscle damage in the same order as their pharmacological potency, e.g. L-quisqualate >L-glutamate >L-cysteine sulphonate >L-aspartate and L-kainate. Low Ca saline slowed the development of degeneration by glutamate, while high Ca saline accelerated the degeneration. 100mM K saline did not promote muscle fiber damage. Denervation, which induces supersensitivity in locust muscles, enhanced the degeneration initiated by glutamate. One possible mechanism for the cytotoxicity of glutamate was tested by measuring the increase in ^{45}Ca in the muscles. Intracellular Ca increased in muscles treated with Con-A and glutamate, an increase which was 10 times greater than in muscles treated with only glutamate. A similar increase in intracellular Ca, initiated by KA, could be partially responsible for the selective degeneration of vertebrate neurons with glutamate receptors.

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44.11 THE RELEASE AND NEOSYNTHESIS OF GLU ARE INCREASED IN EXPERIMENTAL MODELS OF HEPATIC ENCEPHALOPATHY. F.Moroni,G.Lombardi*,D.Pellegrini*,C.Paparozzi*,C.Cortesini*.Dept.of Pharmacology and Dept.of Surgery University of Firenze.Firenze ITALIA.

In order to invenstigate the role of aminoacid mediated neurotransmission in the pathogenesis of hepatic encephalopathy,we studied the content,synthesis and release of Glutamate (GLU), GABA and Glutamine (GLN) in rats with a surgically constructed porto-caval anasthomosis (PCA) or after ammonium acetate administration (7 mmoles/Kg).

The synthesis of GLU and GABA was studied by infusing to the animals ^{13}C-glucose and by measuring the amount of endogenous and $^{13}C_2$-labelled aminoacids with mass-fragmentography.(Moroni et al. J.P.E.T.270,870-877,1978).The release of Glutamate and GABA was monitored by applying cortical cups and by measuring aminoacid output as previously described.(Moroni et al.Naunyn-Schmiedeberg Arch.Pharmacol.316,235-239,1981).

Four weeks after PCA,the content and the synthesis of GABA were not modified in the striatum,wile in the cortex and hippocampus of the same animals the incorporation of $^{13}C_2$ derived from ^{13}C-glucose into GLU was approximately doubled.In these animals the concentration of GLN in the blood and in the brain increased by 90% and by 300% respectively.

Ammonium acetate (7 mmoles/Kg; i.p.; 30 min) doubled the incorporation of $^{13}C_2$ into the molecule of GLU and increased by 90% the release of the aminoacid from the parietal cortex.

Taken together the results suggest an increased availability of GLU at the receptor level in the two experimental models of hepatic encephalopathy studied. Since an increased function of GLU releasing neurons may cause convulsions, spreading depression and eventually gliosis and neuronal degeneration, it is possible that the increased activity of the glutamatergic system play a role in the pathogenesis of hepatic encephalopaty.

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44.12 CATECHOLAMINES (CA) IN BLOOD OF RATS WITH PHEOCHROMOCYTOMA (PHEO) N. Alexander, S. Yoneda* and N. Vlachakis* Univ. of So. Cal. Sch. of Med. Los Angeles, CA 90033

We reported that human and rat red blood cells (RBC) contain CA and CA metabolites (Life Sci. 29:477, 1981). We now report that rats with chronically elevated norepinephrine (NE) and dopamine (DA) also have high concentrations of CA in RBC. The New England Deaconess Hospital (NEDH) rat develops a PHEO after scapular implant of tumor fragment. Groups (n=9) of adult, female NEDH rats, with and without tumor implant, were used for weekly measurements of tail blood pressures and tail vein CA. The first rise in pressure and plasma NE occurred 3 weeks after tumor implant, viz., +9 mm Hg ($p<0.01$) and +1.1 ng/ml (<0.05), respectively. Controls (sham implant) showed an insignificant change of +4 mm Hg and no change in NE. Tail pressure and plasma NE continued to rise in PHEO rats and, in those still living 6 weeks after implant, mean increases were 20 mm Hg (<0.001) and 2.5 ng/ml (<0.01) respectively. Plasma DA also rose significantly (1.0 ng/ml) and in parallel with plasma NE in PHEO rats whereas plasma epinephrine did not change. RBC NE and DA concentrations were increased simultaneously and to the same extent as those of plasma in PHEO rats. Thus from 3 to 6 weeks after tumor implant RBC NE and DA were significantly higher than in controls. Starting 3 weeks post implant, 2 major metabolites, normetanephrine and dihydroxyphenylethyleneglycol, also rose continuously in plasma of PHEO rats. In some rats, 4 to 6 weeks after implant, an indwelling aortic catheter was used to measure mean arterial pressure (MAP) and to sample blood at 3 intervals, AM, noon and PM, over a period of 12 hours in free moving rats (n=10). In PHEO rats plasma NE declined 1.2±.5 ng/ml between AM and noon (<0.05). Their RBC NE showed a parallel but insignificant decline, 0.80±.4 ng/ml (NS) indicating a slower loss of NE from RBC than from plasma. Plasma and RBC NE and DA were significantly higher in PHEO than in control rats at each time interval yet PHEO MAP was consistently only 8-9 mm Hg higher (NS). Thus unrestrained, undisturbed PHEO females did not have a significant MAP elevation. Summary: The NEDH PHEO rat is uniquely suited for studies of chronically elevated circulating CA and CA metabolites and this study showed that RBC as well as plasma of female PHEO rats contain high concentrations of both NE and DA within 3 weeks of tumor implant. Restrained, but not free moving, PHEO rats had elevated arterial pressure. A decline in PHEO RBC NE lagged behind that of plasma NE.

45.1 THE FROGMOUTH AND THE EAGLE: A COMPARISON OF OCULOMOTOR STRATEGIES. Josh Wallman and John D. Pettigrew. National Vision Research Institute, 386 Cardigan St., Carlton, 3053 Victoria, Australia; City College, City Univ. of N.Y., New York, NY 10031.

Among birds, the location of the eyes and the geometry of the foveae probably represent a variety of compromises between the need for frontal, binocular, vision and the need for lateral, panoramic, vision. As a start in understanding the implications of these different compromises, we have compared two predatory birds which both use binocular vision for hunting, but which differ in their use of panoramic vision. The Little Eagle (Hieraaetus morphroides) is a diurnal predator whose retina contains two foveae: a binocular fovea looking frontally which subserves stereopsis and a monocular fovea used in lateral vision. The Tawny Frogmouth (Podargus strigoides) is a nocturnal predator, whose eyes contain a single, frontally directed, binocular fovea. It hunts from close to the ground, so prey may appear anywhere within a large part of its visual field; when threatened it adopts a camouflage posture. Both of these situations seem to favor lateral vision. We report here differences in oculomotor behavior, specifically in the position of primary gaze and in the yoking of saccades, consistent with the different habits of the two birds.

Scleral search coils were implanted under the conjunctiva near the limbus. The coils were calibrated with reference to retinal landmarks seen through an ophthalmoscope; these were compared to more complete retinal maps made on anesthetized animals.

The frogmouth showed a pattern of eye movements consistent with the competing demands of lateral and frontal vision; most of the time the eyes remained near the lateral extreme of the oculomotor range observed, thereby maximizing the field of vision. In this position the region of binocular overlap was eliminated. Saccades were infrequent and usually disjunctive. Large convergent saccades (up to 24 deg) brought the visual axes into alignment, presumably to permit binocular vision.

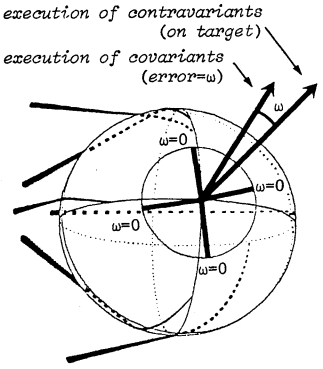
In contrast, the eagle tended to keep its eyes closer to the medial edge of its oculomotor range. In the primary position of gaze, its the binocular foveae were 24 deg divergent (exactly straddling the region of binocular overlap), so that the foveae were within the binocular field, but just barely so. Saccades were very frequent and most were approximately conjugate, although unequal saccades in the two eyes often caused the gaze to be directed more laterally in one eye at a time.

Our data indicate that there is a spectrum along which species vary in the degree of saccadic yoking. The frogmouth, in which conjugate saccades are rare, lies near one end, while the eagle lies somewhere near the middle; primates, to whom Hering's Law applies much more strictly, represent the other extreme.

45.2 TENSOR NETWORK THEORY APPLIED TO THE OCULOMOTOR SYSTEM. CNS ACTIVITY EXPRESSED WITH NATURAL, NON-ORTHOGONAL COORDINATES. G. Ostriker, A. Pellionisz and R. Llinas. Dept. Physiology and Biophysics, New York Univ. Med. Ctr., 550 First Ave., NY 10016.

Nature often provides inherently oblique systems of coordinates, where Cartesian conventions are not valid. The extra-ocular motor system is an example of a geometry in which the intrinsic frame of reference is clearly non-orthogonal. Previous models (Robinson, Boeder) have defined the positional parameters of the extra-ocular muscles. It is apparent that a general approach to coordinate systems, such as tensor analysis, is needed. Expressing the above models with this method, applicable to any coordinate system, the implications of non-orthogonality and an overcomplete number of axes can be revealed naturally.

While an eye movement is a physical invariant, we describe it vectorially in "oculomotor hyperspace" by either covariant or contravariant components. By definition, movement arises through the physical summation of contravariant vector components. However, if the CNS were to relay intention (covariant) vectors directly to the extraocular muscles, the degree of accuracy in the production of intended movements would be a measure of the orthogonality of the system. For saccadic eye movements we work with two coordinate systems, one based upon the actual length changes of the six extraocular muscles and their axes of rotation, and another which is a projection of the first system onto a tangent plane. We characterize saccadic eye movements (from primary, secondary, and tertiary positions) by both covariant and contravariant components. Allowing covariant vectors to be relayed to the muscles (as if they were contravariants), this movement will differ from the original direction by an "error" angle ω, as shown.



Orthogonality would result in eigenvectors (defined by ω=0) in all directions. Our results show that the execution of covariants leads to an error in every direction except for the four eigenvectors of the system. Supported by Grant NS13742.

45.3 ACTIVITY OF ABDUCENS AND OCULOMOTOR NEURONS DURING SYMMETRICAL VERGENCE EYE MOVEMENTS. Lawrence E. Mays and John D. Porter, Departments of Physiological Optics, Physiology & Biophysics and the Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294

There is substantial evidence that the control systems for conjugate and vergence eye movements are relatively independent. Some neurons in the midbrain near the oculomotor nucleus have a pure vergence signal (Mays, Neurosci. Abstr. 7, 1981) while other cells in the pons carry a horizontal conjugate signal only (Mays, ARVO, 1982). Experiments by Keller (Vision Res. 13, 1973) indicate that horizontal rectus motoneurons combine conjugate and vergence signals. Nonetheless, the activity of neurons in the oculomotor and abducens nucleus was re-examined because of: 1) the discovery of abducens internuclear neurons which should carry a conjugate signal only, 2) the existence of oculomotor internuclear neurons, and 3) the fact that earlier experiments used asymmetrical vergence movements which appear to have both conjugate and vergence components.

Extracellular unit recordings were made in three rhesus monkeys trained to look at visual targets for a liquid reward. The horizontal and vertical positions of both eyes were measured using the search coil technique. Symmetrical vergence movements were elicited by changing binocular disparity using a mirror stereoscope or by changing both disparity and accommodation with far and near targets.

In agreement with earlier work by Keller, most abducens neurons appear to carry both conjugate and vergence eye movement signals. A small proportion of abducens units has the purely conjugate signal expected of abducens internuclear neurons. Some abducens units have conjugate and vergence signals which would not be expected of either internuclear or lateral rectus motoneurons. Most oculomotor neurons which have an on-direction appropriate for the medial rectus muscle combine the conjugate and vergence signals. A small proportion clearly has a conjugate signal but no vergence signal.

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45.4 LOCALIZATION OF NEURONS PROVIDING AFFERENT AND EFFERENT INNERVATION OF MONKEY EXTRAOCULAR MUSCLES. J.D. Porter, B.L. Guthrie*, & D.L. Sparks, Dept. of Physiology & Biophysics/Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

Recent studies (Buttner-Ennever and Akert, J. Comp. Neurol. 197, '81; Spencer and Porter, J. Comp. Neurol. 198, '81) concerned with the localization of motoneurons innervating primate extraocular muscles (EOM) have made it clear that the classical scheme of organization is incomplete and, in some cases, incorrect. Moreover, the proprioceptive innervation of monkey EOM's remains uncharted. As a prelude to our physiological studies of the EOM proprioceptive system, retrograde horseradish peroxidase (HRP) techniques were used to identify and localize muscle afferent and efferent cell somata. Individual EOM's of adult rhesus monkeys were isolated and injected with 30-50 µl of a 10% HRP solution. Following 24 hour survival and paraformaldehyde/glutaraldehyde fixation, sections were cut and processed for the histochemical demonstration of HRP. Examination of all brainstem motor nuclei served as the control for our injections.

Our data extend the findings of recent HRP studies to include all muscles having an action upon the globe. Motoneurons lying within the oculomotor nucleus exhibited a more complex organization than that described by Warwick (J. Comp. Neurol. 98, '53). Medial (MR) and inferior rectus (IR) representations were in accordance with the contemporary HRP data. Superior rectus (SR) motoneurons were distributed along the medial aspect of the contralateral nucleus, with the predominate representation at caudal to mid-nuclear levels. SR motoneurons were also clustered among the small MR and IR motoneurons in the dorsomedial portion of the nucleus. Inferior oblique motoneurons filled the gap lateral to the SR group and ventral to MR and IR at their respective levels. Superior oblique motoneurons were confined to the trochlear nucleus contralateral to the injection site. Motoneurons innervating the lateral rectus muscle were found in the abducens nucleus proper and in the ventral abducens nucleus, as noted by Spencer and Porter ('81). Consistent with data in the cat (Porter and Spencer, J. Comp. Neurol. 204, '82), neurons subserving sensory innervation of the extraocular muscles were confined to the semilunar ganglion. Section of the ophthalmic division of the Vth nerve prior to HRP placement selectively disrupts ganglion labeling while motor innervation remains intact. Thus EOM afferent fibers course centrally along a path distinct from the motor supply. The separation of fiber types allows chronic deafferentation of the EOM's to be performed in order to assess the role of muscle proprioception in the control of eye movements (Guthrie et al., Soc. Neurosci. Abstr., '82).

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45.5 ROLE OF EXTRAOCULAR MUSCLE PROPRIOCEPTION IN EYE MOVEMENTS STUDIED BY CHRONIC DEAFFERENTATION OF INTRA-ORBITAL STRUCTURES. B.L. Guthrie*, J.D. Porter and D.L. Sparks (SPON: J. Smythies). Dept. of Physiology & Biophysics/Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294

The extraocular muscles (EOM) of monkeys contain well-developed proprioceptors but their contribution to eye movement remains a mystery. Our anatomic studies show that, in monkey, centrally directed fibers from these receptors pass through the first division of the trigeminal nerve (Porter et al., Soc. Neurosci. Abstr. 1982) and at this location are isolated from motor fibers to EOMs. Taking advantage of this fact, an intracranial procedure was used to chronically deafferent the EOMs by sectioning the ophthalmic nerve bilaterally.

Conjugate and disjunctive eye movements were studied before and after surgery to delineate the role of proprioception. A major goal was to determine whether or not eye position information exists as a central 'corollary discharge' in the absence of proprioception.

Following deafferentation spontaneous saccades in the dark and light were similar to control movements. Saccadic eye movements to continuously present and remembered targets were normal. In the stimulate-compensate paradigm of Mays and Sparks (Science 208, 1980) deafferented monkeys made compensatory saccades to remembered targets. This indicates that EOM proprioception is not essential for spatial localization of visual targets.

After surgery binocular fixation of visual targets was interrupted by slow adduction of the non-dominant eye while the dominant eye remained on target. Monocular fixation of a visual target was accompanied by slow adduction of the nonviewing eye while the viewing eye (right or left eye) remained on target.

No fundamental deficits in smooth pursuit were identified except that the above mentioned monocular 'drift' was superimposed on pursuit movements.

Deafferented monkeys performed vergence tasks poorly. Although an appropriate initial vergence response was made to stimuli with crossed or uncrossed disparity, the resultant vergence angle was not maintained due to the medial 'drift' of the non-dominant eye.

In the absence of proprioception: a) a central 'corollary discharge' is used for spatial localization of targets; b) conjugate eye movement signals are normal; and c) maintenance of binocular alignment during binocular or monocular target fixation is impaired. The hypothesis that EOM proprioception is a feedback signal used to stabilize binocular alignment after disparity or accommodative vergence eye movements is being investigated.

(Supported by EY01189, P30 EY03039 and F32 EY005651).

45.6 THE FINE STRUCTURE OF OCULOMOTOR MOTONEURON ACTIVITY DURING SACCADES. H. P. Goldstein* and D. A. Robinson. Wilmer Institute, The Johns Hopkins University, Baltimore, MD.

The waveform of the firing rate of oculomotor motoneurons during saccades has been grossly described as a per-saccadic, high frequency burst of firing (the pulse) abruptly falling to a post-saccadic, tonic firing rate (the step). However, details of the per-saccadic discharge rate and the per- to post-saccadic transition were not known. Knowledge of these details is required to test models of the oculomotor plant during saccades. Specifically, hysteresis has been a controversial issue: Does the steady-state firing rate at a given position depend on the path the eye took in getting to that position? Claims have been made for and against hysteresis. It was possible that hysteresis was confused with slow changes in the post-saccadic rate arising from compensation of long time constant plant elements. We believe we have resolved this issue.

Single units were recorded in the abducens nucleus of monkeys (Macaca mulatta) trained to follow a visual target. Eye position was measured by the search coil technique. Behavior was averaged over many, nearly identical saccades. We had the monkeys make repeated saccades from an eccentric position (+20 or -20 deg) to primary position. The eccentric gaze was held for three seconds giving adequate time for long time constant viscous plant elements to reach steady state. Because of the possible reflection of these long time constants in the neural signal, we acquired data for three seconds after the target stepped. The per- to post-saccadic transition (from the pulse to the step) was not abrupt as previously held, but instead reached the final tonic rate with an exponential time course: an initial exponential with a short time constant followed by a variable exponential tail with a longer time constant. Typically the initial exponential had a time constant of 90 milliseconds and accounted for 16% of the per- to post-saccadic transition. For the saccades ending at identical positions but coming from different directions, the final firing rates were also identical thus showing no signs of hysteresis.

45.7 STRUCTURE FUNCTION CORRELATION OF ABDUCENS MOTOR AND INTERNUCLEAR NEURONS IN RELATION TO EYE MOVEMENTS: AN INTRACELLULAR HRP STUDY IN THE ALERT SQUIRREL MONKEY. A. Strassman*[1], R.A. McCrea[2] and S.M. Highstein[1]. Albert Einstein College of Medicine[1], Bronx, New York 10461 and University of Chicago, Chicago, Illinois 60637

Male squirrel monkeys (600-800gms) were implanted with scleral search coils to measure eye movements with the magnetic field technique. A modified bolt was cemented to the skull for head stabilization and animals were seated in a primate chair. A recording chamber was implanted over the cerebellum and horseradish peroxidase (HRP) loaded glass microelectrodes advanced into the brain stem. Eye movements were either spontaneous, pursuit or rotation induced nystagmus. The usual recording and injection sites were intra-axonal. After characterization and recording of physiological activity (spike frequency) in relation to gaze, twelve abducens internuclear and four abducens motoneurons were injected with HRP. After 24-30 hour survivals, a modified diaminobenzidine-cobalt intensified protocol was used to develop the HRP reaction product.

Discharge patterns of both classes of neuron were burst-tonic or tonic with an ipsilateral on-direction. Rate-position plots yielded k values of motoneurons ranging from 10-14.5. K values of internuclear neurons ranged from 7-24. Both classes of cells usually burst for on-direction and paused for off-direction saccades or quick phases of nystagmus. Complete soma-dendritic plus axonal fills were not uncommon. Neuronal soma size and location was overlapping but the smaller internuclear neurons tended to have a caudal collateral. Dendritic fields of both classes of cells were completely overlapping and confined within the cellular borders of the nucleus. Dendrites of motoneurons, in contrast to internuclear neurons, were more numerous, thinner, more highly tapered and more branched, as in the cat (Highstein et al., J.C.N., in press). There were no axon collaterals of either class of neuron within the abducens nucleus but one motoneuron had an axon collateral which terminated in the abducens nerve below the nucleus. Internuclear neurons terminated in the medial rectus subgroup A of the IIIrd nucleus. Approximately one third also terminated in and around the median longitudinal fasciculus both in front of and behind the abducens nucleus; one third lacked the caudal collateral and terminated in front of the abducens; and the remaining third traveled to the oculomotor nucleus without collaterals.

These studies support the role of abducens moto- and internuclear neurons in the production of conjugate horizontal gaze.

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45.8 THE LOCATION AND PROJECTIONS OF SQUIRREL MONKEY VESTIBULAR NEURONS WITH HORIZONTAL EYE POSITION SENSITIVITY. S.M. Highstein, R.A. McCrea and A. Strassman* (SPON: J.M. Goldberg). Dept. Neuroscience, Albert Einstein Coll. Med., Bronx, NY 10461; and Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

The purpose of the present study was to identify the location and axonal projections of vestibular neurons in the alert squirrel monkey whose activity was related to eye movements in the horizontal plane. Squirrel monkeys were prepared for recording by implanting a chamber over the cerebellum so that microelectrodes filled with a solution of HRP could be inserted into the brainstem. During the experiment, the monkey's head was restrained by fixing a chronically implanted bolt to a primate chair positioned on a vestibular turntable. Eye movements were measured with the magnetic search coil technique. In a few experiments, stimulating electrodes were chronically positioned on the oval and round windows for electrical stimulation of the vestibular nerve.

A total of 17 vestibular axons whose physiological activity was related to horiontal eye position were injected and recovered. Three of these neurons were also activated at monosynaptic latencies (<1.1 msec) following electrical stimulation of the vestibular nerve. In addition to horizontal eye position, the firing rate of the injected vestibular axons was also related to eye velocity during smooth eye movements. Most of the axons paused during saccades in all directions, except for saccades with a horizontal component in the on direction. Eight of the injected cells had axons coursing rostrally in the ascending tract of Deiters (ATD), and were located in the ipsilateral ventral lateral vestibular nucleus (VLVN) or the lateral part of the medial vestibular nucleus (MVN). Most of the ATD axons projected to the oculomotor nucleus without giving rise to collaterals to other areas. However, two axons gave rise to collaterals which terminated ventromedial to the ipsilateral abducens nucleus, and one gave rise to a collateral which terminated in the reticular formation rostral to the abducens. Six of the injected axons originated from cells in the MVN or the VLVN, crossed the midline, and terminated in the contralateral abducens nucleus and MVN. In addition, these axons had collaterals which ascended and descended in the contralateral MLF. The descending collaterals terminated in the prepositus nucleus, the medullary reticular formation, and the midline raphe nuclei. The ascending collaterals terminated in the dorsal pontine reticular formation, and could be followed to the oculomotor nucleus. The results of these experiments demonstrate that many of the primate vestibular neurons with horizontal tonic eye position sensitivity project to medial or lateral rectus motor nuclei, and that some of these neurons receive direct inputs from the vestibular nerve. The collateral projections of these neurons suggest that the prepositus, the dorsal pontine reticular formation, and the medullary raphe may play an important role in horizontal vestibuloocular reflexes.

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45.9 THE LOCATION AND COLLATERAL PROJECTIONS OF SQUIRREL MONKEY VESTIBULAR NEURONS WITH VERTICAL EYE POSITION SENSITIVITY. R.A. McCrea, A. Strassman* and S.M. Highstein. Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637; Dept. Neuroscience, Albert Einstein Coll. Med., Bronx, NY 10461.

The location and axonal projections of vestibular neurons whose activity was related to vertical or oblique eye movements was studied by injections of HRP into the axons of 25 vestibular neurons which had been physiologically characterized in the alert squirrel monkey. In a few experiments, stimulating electrodes were chronically implanted on the oval and round windows for electrical stimulation of the vestibular nerve; and five of the injected vertical eye movement related axons were activated at monosynaptic latencies following stimulation of the vestibular nerve. The firing rate of all of the injected axons was related to eye position, and most tended to pause during saccades. Three major groups of cells were injected.

One group of axons (N=8) which responded maximally during downward oblique eye positions arose from cells in the lateral part of the medial vestibular nucleus (MVN) and the medioventral lateral vestibular nucleus (VLVN). These axons ascended in the MLF and terminated in the contralateral trochlear nucleus (IV) and lateral aspect of the oculomotor nucleus (III). In addition, some axons had collaterals which terminated in the dorsal raphe nucleus (DR) the interstitial n of Cajal (IC), and in the region of the MLF. One neuron gave rise to a collateral which terminated in the contralateral abducens nucleus (Abd). Caudal branches of these axons terminated primarily in the region of Roller's nucleus (RN) and the caudal medullary raphe.

A second group (N=3) arose from cells in the caudal superior vestibular nucleus, and responded maximally during upward oblique eye positions. The axons of these cells ascended and terminated in ipsilateral IV, the lateral part of III, and IC. Two cells gave rise to collaterals which terminated in the ipsilateral pontine and medullary reticular formation, and one cell had a collateral which terminated in the ipsilateral Abd.

The third group of axons (N=14) arose from cells in the VLVN and the lateral aspect of the MVN, and responded maximally during upward eye positions. These axons ascended in the contralateral MLF and terminated in the medial aspect of the contralateral III and in the IC. One cell terminated bilaterally in III. Caudal branches of these axons terminated in the medullary reticular formation, RN and in the medullary raphe nuclei.

The results of this experiment demonstrate that many primate vestibular neurons with vertical eye position sensitivity receive direct inputs from the vestibular nerve, and project to extraocular motor nuclei. The collateral projections of these cells suggest that the medullary raphe nuclei and RN may play an important role in vertical vestibuloocular reflexes, and that the abducens nucleus receives inputs from some vertical vestibular neurons.

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45.10 MESENCEPHALIC NEURONS THAT DISCHARGE FOR TARGET MOVEMENTS, SACCADES AND EYE POSITION. C.R.S. Kaneko and A.F. Fuchs. Regional Primate Research Center and Dept. Physiol. & Biophys., Univ. of Wash., Seattle, Washington 98195

Our preliminary injections of horseradish peroxidase into the omnipause neuron region of the cat revealed a large afferent input from a restricted midline mesencephalic area. Since omnipause neurons are thought to be an essential link in the production of saccades, we wanted to ascertain whether this mesencephalic region contained neurons whose discharge might convey appropriate oculomotor signals. During the course of single unit recordings from this area in alert, trained animals, we discovered a group of neurons that discharge in relation to both eye and target movement.

In two cats, a region rostral to the oculomotor nucleus and medial to the retroflex bundle contained neurons that discharged with a sporadic tonic rate that was related to vertical eye position in about one-half of the population. Slopes of the rate/position relation ranged from 0.5 to 2.1 spikes/deg. All neurons also showed a tonic or phasic increase or decrease in discharge for vertical saccades in a preferred direction although some sensitivity to pure horizontal movements was seen. The preferred direction for saccades could be in the same or opposite direction to the eye position preferred direction. In contrast, target movements in the preferred direction for saccades elicited an increase in firing rate. This increase was maintained until the saccade-related discharge, after which the firing rate returned to that appropriate for the newly obtained eye position. The saccade-related discharge was independent of target movement since it occurred during spontaneous saccades. Target-related discharge was independent of eye movement since it occurred when the target was moved and the animal did not track the target. Since these neurons continued to discharge while a retinal error was present following a target step, we have tentatively termed them error cells.

We have recorded similar neurons in the mesencephalon of a single monkey. These neurons displayed a wider variety in their discharge characteristics: although nearly all had eye position sensitivity (range 0.9-4.0 spikes/deg) a few showed none; and while most had high tonic rates of discharge, some had none. The discharge in response to target movements and saccades was less reliable but usually synergistic. In addition some neurons discharged most vigorously for oblique target/eye movements.

45.11 PROBABLE INHIBITORY BURST NEURONS IN THE MONKEY. C. A. Scudder, T. P. Langer*, and A. F. Fuchs. Regional Primate Center and Dept. of Physiol.& Biophys., Univ. of Wash. Seattle, WA 98195.

In the cat, Hikosaka and his collegues have identified a group of neurons immediately caudal to the abducens nucleus which discharge a burst of spikes for ipsilateral saccades and make monosynaptic inhibitory connections with the contralateral abducens nucleus. They have been appropriately named Inhibitory Burst Neurons (IBNs). In the monkey, we have recorded burst neurons in a location comparable to that of cat IBNs. Three lines of evidence suggest that these are IBNs in the monkey. (1) Horseradish Peroxidase (HRP) injected into one abducens nucleus retrogradely labeled cells just ventrocaudal and slightly medial to the contralateral abducens nucleus. We verified that this was the location where burst neurons were recorded by placing electrolytic lesions at appropriate recording sites, injecting HRP in the same monkey, and comparing adjacent Nissl and HRP-processed tissue sections. (2) Microstimulation (10 μA) near putative IBNs yielded eye movements and EMG responses appropriate for a crossed inhibitory connection. During fixation, stimulation caused the eyes to move ipsilaterally. Moreover, responses from EMG electrodes in the contralateral lateral rectus muscle were of opposite sign, and of 0.5 ms longer latency, than responses elicited by contralateral abducens nucleus microstimulation. (3) As in the cat, cells discharged a burst of spikes for ipsilateral saccades and little or no burst for contralateral saccades. That is, for a cell with a crossed inhibitory connection, the burst was functionally appropriate.

A quantitative analysis of 17 neurons revealed that the onset of discharge in monkey IBNs filled the range from short-lead to long-lead. Only about one-fourth were classical short-lead burst neurons (5.1-10 msec latencies), for which the number of spikes was strongly correlated with saccade size, as was burst duration with saccade duration. The discharge of the remaining burst neurons (10-200 ms latency) included a compact burst ($\geq$ 300/sec) whose onset immediately preceeded the saccade. For this portion, burst parameters were also correlated with saccade parameters but more weakly than for short-lead burst neurons.

45.12 HORIZONTAL OR GLOBAL GAZE PALSY INDUCED BY KAINIC ACID LESIONS IN THE PONS OF THE MONKEY. V. Henn*, W. Lang*, K. Hepp* and H. Reisine (SPON: M. B. Bender). Depts. of Neurology and Pathology, Univ. Hosp., Dept. of Physics, ETH; Zürich, Switzerland.

Rhesus monkeys were prepared for chronic single unit recordings in the paramedian pontine reticular formation (PPRF). Single unit activity in this area has been related to rapid eye movements. Long lead burst signals have been located in the more rostral parts in contrast to the short and medium lead as well as burst-tonic (i.e. signals related to eye position also) activity found in more caudal parts. Small injections of kainaic acid were placed selectively into rostral or caudal areas to observe their differential effects. Such injections lead to preferential loss of cells leaving fiber systems mostly unaffected. Histology shows a rather sharply limited area around the injection site with complete loss of neurons and invasion of microglia.

Unilateral PPRF injections lead to neurological deficits which are identical to those after electrolytic lesions with loss of all rapid eye movements towards the ipsilateral side (Bender and Shanzer, The Oculomotor System, N.Y., 1964).

Bilateral rostral PPRF lesions lead to a complete loss of rapid eye movements in the horizontal plane only, leaving vertical saccades intact.

Bilateral caudal PPRF lesions lead to a total loss of rapid eye movements in all directions.

In all cases of unilateral or bilateral lesions slow eye movements induced by vestibular stimulation were intact in all directions. With bilateral lesions, pursuit movements could be elicited, and the eyes could be held in eccentric positions of gaze. The experiments support the hypothesis that on a premotor level the generation of rapid and slow eye movements are independent from each other; that the velocity-to-position integrator necessary to hold the eyes in eccentric gaze positions is located outside of the PPRF; that the caudal PPRF plays an important role in coordinating and controlling the occurrence of rapid eye movements in all directions although the immediate premotor apparatus for generating rapid vertical eye movements is located in the rostral mesencephalon.

45.13 SINGLE UNIT RECORDING WITH FREELY MOVING HEAD: A NEW MICRODRIVE PRINCIPLE. B. Merker* and J.D. Schlag. Dept. of Anatomy and B.R.I., U.C.L.A., Los Angeles, CA 90024.

In the past the technique of single unit recording in behaving animals achieved precision in electrode control at the price of highly restrictive behavioral conditions. The size, weight, and mode of advancement of conventional microdrives prevent them from being mounted on the unrestrained head of animals. This limitation has been overcome in a new microdrive system which allows electrodes to be moved with micron accuracy and full compliance on the unrestrained head without impeding any of its movements.

A mechanical transducer of novel design, weighing 8g and measuring 8x10x41mm, converts one full turn of rotary input motion to 1.09μm of linear output motion over a range of 20mm of continuous fine travel (unlimited gross travel). Mounted on a conventional chronic well, its low input torque requirements allow it to be driven remotely by a fine and flexible stainless steel wire, suspended from a mount at a convenient distance. From there the wire, in turn, can be rotated either by mechanical means or via an electric motor. The system currently in use on cat and monkey permits the animal to move its head with complete freedom during single unit recording, its trunk alone being restrained.

The low mass of the head-mounted transducer means that even rapid and violent head movements transmit no significant force to the electrode. Single unit records are therefore stable, well isolated units with a signal-to-noise ratio of 3:1 or better being held through bouts of struggling, head shaking, and experimenter manipulations such as alternately fixing and freeing the head.

The system works to best advantage in combination with a search-coil system for monitoring movement: the same inducing magnetic fields that yield a gaze signal from a scleral search coil yield a head position signal from an equivalent coil permanently attached to the head. In addition, wire loops fastened to ear or limb may be used to obtain information on ear position and the timing of limb movements.

The performance of the system will be demonstrated on film, and via single unit and behavioral records obtained in the course of microelectrode exploration of the subthalamic region (zona incerta, fields of Forel, and prerubral field) in relation to the behavior of eyes, head, and paws.

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46.1 SINGLE-UNIT RECORDINGS FROM A VOCAL CONTROL NUCLEUS OF THE SINGING MOCKINGBIRD. J. S. McCasland and M. Konishi. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

We have developed a new technique for recording from single neurons in song system nuclei of the freely-behaving, singing mockingbird. The method employs an X-Y microdrive designed by our machinist Herb Adams. This device of approximately two grams is constructed so as to allow multiple electrode tracks over a 4 x 4 mm area, thus allowing a nucleus to be mapped for single unit properties during song. This confers a special advantage in the song system because its nuclei are anatomically discrete, with boundaries which can be physiologically defined. Thus, in principle such mapping can be done over long periods of time without the need for sacrificing birds to verify track locations. The chronically implanted microdrive is sufficiently stable so that units can be isolated and held for periods up to several hours.

Our preliminary data show that many cells exhibit premotor activity for all mockingbird song elements, and do not respond to those same song elements presented as auditory stimuli. Some of these cells have relatively constant pre-sound latencies for the various elements, while others show long-latency "anticipatory" activity at the initiation of song and between elements of a long song bout.

A few cells show more selective premotor activity, producing highly stereotyped bursts of spikes for only a few song elements. Thus far we have not found examples of such cells which are also responsive to playback of the sounds for which they produce motor activity. At least some of the cells which are responsive to playback are inhibited during singing, as predicted from our multiple-unit data.

Interaction between motor output and auditory feedback is known from behavioral experiments to be necessary for the motor phase of song learning. The ability to examine this interaction at the cellular level should enable us to assess the neurophysiological correlates of song learning in the various nuclei of the vocal control system.

46.2 PATTERN GENERATION BY DELAYED EXCITATION IN PARALLEL WITH RECIPROCAL INHIBITION. P.A. Getting. Dept. of Physiol. Biophys., Univ. of Iowa, Iowa City, IA. 52242.

The central pattern generator (CPG) network underlying escape swimming of the mollusc Tritonia diomedea consists of four premotor interneuron pools termed cerebral cell 2 (C2), dorsal swim interneurons (DSI), and two subgroups of ventral swim interneurons (VSI-A & VSI-B). During a swim, bursts in DSI alternate with coactive bursts in both VSI-A and VSI-B. Mapping of the monosynaptic connectivity among the four CPG interneuron types revealed that the synaptic basis for pattern generation by this network was reciprocal inhibition between DSI and VSI paralleled by delayed synaptic excitation from DSI to C2 to VSI. Delayed excitation of the VSI group was mediated by two distinct mechanisms. For VSI-A, delayed excitation resulted from the integrative properties of the multicomponent inhibitory-excitatory synapse from C2 to VSI-A. The initial action of this dual action synapse was inhibition, but after a delay of 1-1.5 sec, this synapse converted to excitation resulting in the delayed burst in VSI-A. The mechanism of delayed excitation in VSI-B was distinctly different and relied upon the interaction of synaptic excitation with tha activation of a transient outward potassium current (A-current). The VSI-B had a deep resting potential (-60 mV) which removed inactivation of A-current. The VSI-B responded to injected depolarizing currents with a long delay of 0.4-4.0 sec to the first spike. Once repetitive firing started, the spike frequency accelerated to a steady state level proportional to the injected current. Voltage clamp of VSI-B revealed that both the long delay and the acceleration in spike frequency could be accounted for by the activation and subsequent inactivation of A-current upon depolarization. The VSI-B received only synaptic excitation from C2, thus the delay in the onset of VSI-B bursts during a swim resulted from the interaction of synaptic excitation with the kinetic properties of A-current intrinsic to the VSI-B. Reciprocal inhibitory networks will produce repetitive bursts only if some additional properties are included. Previously proposed mechanisms include post-inhibitory rebound, synaptic fatique, and accumulative refractoriness. The reciprocal inhibitory network of the Tritonia swim system uses a new mechanism of delayed, synaptically mediated excitation.

46.3 THREE FORMS OF THE TURTLE SCRATCH REFLEX. Lawrence I. Mortin, Joyce Keifer* and Paul S.G. Stein. Dept. of Biology, Washington University, St. Louis, MO 63130.

The red-eared turtle Pseudemys scripta elegans, when low-spinal, displays three different forms of the scratch reflex behavior in response to gentle mechanical stimulation. Biomechanical constraints on the hindlimb and body compel the turtle to use different strategies of limb movements to scratch separate parts of its body. Stroboscopic videotape studies of hindlimb movements and electromyographic recordings were used to analyze the different aspects of each scratch form. The rostral scratch reflex (previously called the turtle scratch reflex; J. Comp. Physiol. 140:287, 1980, 145:477, 1982 and 146:401, 1982) is initiated by stimulation of the shell bridge region. Hip protraction moves the hindlimb rostrally. With the hip held protracted, the knee extends and the dorsum of the foot performs the rub. Knee extensor muscle (FT-KE; femorotibialis) activity occurs during the latter half of hip protractor muscle (VP-HP; ventral puboischiofemoralis internus) activity in the rostral scratch. The pocket scratch reflex is initiated by stimulation of the skin or shell of the pocket region, located between the shell bridge and the hindlimb. The hip is protracted and the knee flexed to bring the limb into the pocket. Then knee extension and hip retraction occur simultaneously as the knee performs the rub. FT-KE activity occurs after VP-HP activity and during hip retractor muscle (HR-KF; flexor cruris, pars flexor tibialis internus) activity in the pocket scratch. The caudal scratch reflex is initiated by stimulation of the shell or skin caudal to the hindlimb. Hip retraction moves the limb caudally. With the hip held retracted, the knee is extended and the heel or side of the foot performs the rub. FT-KE activity occurs after HR-KF activity and before VP-HP activity in the caudal scratch.

A transition zone (TZ) exists at the boundary between two adjacent scratch reflex receptive fields. Within a TZ the turtle's limb has the mechanical ability to use either of the two bordering scratch reflex forms to rub against the stimulated site. Stimulation within a TZ can elicit (1) a pure form of the scratch, indistinguishable from a scratch initiated within the receptive field rostral or caudal to the TZ, (2) a switching scratch behavior, a multicycle scratch in which one cycle of a pure form of scratch is followed by a subsequent cycle of a second pure form of scratch, or (3) a hybrid scratch reflex, such that within a single cycle of scratch there are characteristics typical of two different scratch forms. The smooth and uninterrupted nature of the hindlimb movements during a TZ scratch imply some form of convergence in the controlling elements for the different scratch reflex motor programs. Supported by NIH Grant NS-15049 to PSGS and NIH Training Grant in Neurobiology NS-07071 to LIM.

46.4 CENTRAL PROGRAMS FOR THREE FORMS OF THE TURTLE SCRATCH REFLEX. Gail A. Robertson, Joyce Keifer* and Paul S.G. Stein. Dept. of Biology, Washington University, St. Louis, MO 63130.

Three distinct forms of the scratch reflex are centrally programmed in the turtle spinal cord. The motor program underlying each scratch form has been described for the freely moving limb using electromyography (EMG). Each motor program is elicited by stimulation in a specific receptive field (Mortin et al., this vol.). In the immobilized, low-spinal turtle, identified scratch programs can be recorded from peripheral nerves innervating the hip protractor (HP) muscle VP-HP, the knee extensor (KE) muscle FT-KE, and the hip retractor (HR) muscle HR-KF. For each scratch form, sequences of activation of hip and knee motor neuron pools exhibited in the peripheral nerve recordings (ENGs) are similar to the patterns of muscle coordination observed with EMGs in the moving limb. The receptive field mapped for each central program in the immobilized turtle is identical to that mapped for the corresponding program in the preparation with limb movement. Transition zones exist between adjacent receptive fields in the immobilized turtle, and stimulation in these regions elicits a variety of responses exhibiting many similarities to the transition motor patterns evoked in the preparation with a moving limb.

Intracellular recordings indicate that motor neurons (MNs) of a given pool receive phasic excitatory and inhibitory synaptic drive correlated with the activation of other motor pools. For example, each HP MN receives a Cl-mediated inhibition during the HR phase of a scratch, independent of the receptive field stimullated. The associated antiphasic relationship between HP and HR ENGs is basic to all scratch programs. A feature that differentiates the three scratch forms is the relative timing of the KE activity in the HP activity cycle. In the rostral scratch, KE activity begins late in the HP burst. Both HP and KE activity are terminated at the onset of the HR burst and remain quiescent for its duration. Each HP MN initially exhibits a slow ramp of depolarization and a low frequency discharge. At the onset of KE activity, a marked increase in excitatory drive is reflected in a more rapid membrane potential trajectory and a higher frequency discharge. A single phase of Cl-mediated inhibition is correlated with the subsequent HR burst. In the pocket scratch, KE activity begins after the termination of the HP burst, and is co-active with the first part of the HR burst. Each HP MN receives a single phase of excitatory activation, followed by a Cl-mediated inhibition during the KE phase of the cycle. A second phase of inhibition is correlated with the remainder of the HR burst. These observations show that HP MNs receive timing information linked to the activation patterns of HR and KE MNs characteristic of each scratch form. Supported by NIH Grant NS-15049 to PSGS and NIH Training Grant in Neurobiology NS-07071 to GAR.

46.5 A SIMPLIFIED CIRCUITRY SCHEME FOR THE GASTRIC MILL NETWORK IN LOBSTER TESTED BY PHOTOINACTIVATION OF CELLS. M. Wadepuhl* and A. I. Selverston. Dept. of Biol., Univ. of Calif. San Diego, La Jolla, CA 92093.

One approach to the understanding of complicated networks is to reduce them to a set of basic connections. Predictions made on the basis of such a reduced circuit can then be tested experimentally.

We have reduced the 11 celled gastric mill network in the stomatogastric ganglion to a system of 5 cell groups. We reduced the number of 22 synapses to 6, selecting those, which we and others have determined to be the most important (Russell, D.F.and Hartline, D.K., Science 200:453, 1978; Thompson, R.S., Biol. Cybern 43: 71, 1982; Selverston A.I. et al., Prog. Neurobiol. 7:215, 1976). We have tested this scheme by photoinactivation of groups of cells (Miller, J.P. and Selverston, A.I., Science, 206: 702,1979). The crucial connection appears to be a reciprocal inhibition between Int 1 and the LG/MG-complex. These cells fire alternately and periodically inhibit GM and LPG, which can be viewed as follower cells. Int 1 also excites the DG/AM complex and inhibits the GM. In addition DG exhibits not only plateau potentials (Russell, D.F. and Hartline, D.K., Science, 200:453, 1978), but can be caused to burst in the presence of Octopamine (Wadepuhl, M. and Selverston, A.I., in prep.).

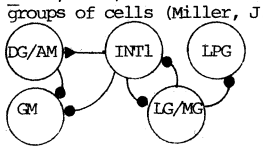


Fig.1 Reduced circuitry Scheme

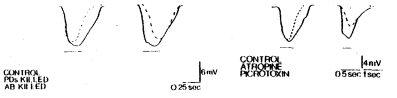
In non-bursting preparations the LPG, GM and Int 1 fire tonically while DG, AM, LG, MG are silent. This allows us to make several predictions:

1) Inactivation of LPG and GM should not alter the pattern.
2) Killing of DG and AM should only reduce the modulation of GM.
3) Inactivation of LG and MG should not effect DG and AM but should cause LPG to go tonic.
4) Killing Int 1 should abolish all patterned activity, except for DG/AM which may continue to burst if there is enough excitory input from outside the ganglion present.
5) Finally the minimal circuit for alternating burst generation should be the Int 1 and LG/MG groups.

We are able to show remarkable coincidence between our experimental results and the predictions. Deviations from our expectations point towards some functional significance of the synapses neglected in the above scheme.

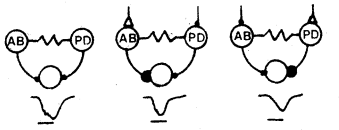
46.6 ELECTRICALLY COUPLED NEURONS RELEASE DIFFERENT TRANSMITTERS RESULTING IN A VARIABLE TIME COURSE SYNAPSE. E. Marder and J.S. Eisen. Biology Dept., Brandeis University, Waltham, MA 02254

The lobster stomatogastric ganglion (STG) contains an ensemble of electrically coupled neurons composed of the 2 PD motor neurons and the single AB interneuron. The PD neurons 1) make cholinergic neuromuscular connections, 2) contain choline acetyltransferase (CAT; E.C.2.3.1.6.; 17.7 ± 1.5 pmole/cell/hr; n=31; activity in 31/33 cells) and 3) evoke increases in G_{K^+} which are blocked by atropine, scopolamine, QNB and TEA but insensitive to picrotoxin. The AB neuron 1) contains no detectable CAT (<1/30 of the activity of a PD neuron; n=9) and 2) evokes rapid increases in G_{Cl^-} plus G_{K^+} which are blocked by picrotoxin but insensitive to atropine, scopolamine, QNB and TEA. The combined AB plus PD-evoked inhibition in postsynaptic neurons can be dissected into its component parts either pharmacologically or using the Lucifer yellow photoinactivation technique as shown by the superimposed tracings below.



bar indicates firing time of presynaptic neuron(s) here and below

The electrically coupled AB & PD neurons act as the pacemaker for the pyloric rhythm of the STG by inhibiting the other pyloric neurons. The functional consequences of the different time courses of AB-evoked and PD-evoked IPSPs are seen below:



resistor-electrical synapse
filled circle-chemical IPSP
triangle-excitatory input
size of filled circle is proportional to amount of transmitter released by that neuron.

Any excitatory or inhibitory input onto either the PD or AB neurons but not both (asymmetric input) will alter the ratio of PD-released to AB-released transmitter and therefore, will alter the ratio of PD-evoked to AB-evoked inhibition in a postsynaptic neuron. Because of the different time courses of PD-evoked and AB-evoked inhibition, this should alter the phase relationships between the AB/PD and postsynaptic neurons.

J.S. Eisen was a Gillette Fellow at Brandeis University. Research support by NIH NS17813 and a Sloan Fellowship to E. Marder.

46.7 ACTIVATION OF ASYMMETRIC INPUTS ONTO ELECTRICALLY COUPLED NEURONS GENERATING A VARIABLE TIME COURSE SYNAPSE: A MECHANISM FOR CHANGING PHASE RELATIONSHIPS BETWEEN NEURONS. J.S. Eisen and E. Marder. Biology Department, Brandeis University, Waltham, MA 02254

The electrically coupled cholinergic PD and glutamatergic AB neurons jointly inhibit the PY neurons of the lobster STG. The following predictions can be made about the phase relationships of the firing times of AB, PD and PY neurons based on the model presented in the previous abstract (Marder & Eisen, above): 1) an increased ratio of PD-released to AB-released transmitter will result in an increase in PD-evoked inhibition. Because the PD neurons evoke the late portion of the combined IPSP (see Marder & Eisen, above), this should prolong the late portion of the IPSP and thus the PY neuron should begin to fire later following a synchronous AB/PD burst than it would under control conditions and 2) a decreased ratio of PD-released to AB-released transmitter should decrease the amount of PD-evoked inhibition and thus, the PY neuron should begin to fire earlier following an AB/PD burst than it would under control conditions.

In order to test this model we activated selectively two different asymmetric inputs into the STG. Dopamine (DA) decreases the ratio of the amplitudes of PD/AB slow wave depolarizations. Therefore, the model would predict that the PY neuron should begin to fire earlier in the presence of DA than under control conditions. This prediction was confirmed in experiments in which the phase relationships between PY and AB/PD neurons were compared under control conditions and in the presence of DA. Stimulation of the IVN through fibers increases the ratio of the amplitudes of PD/AB slow wave depolarizations. The model would predict that the PY neuron should begin to fire later following IVN stimulation than under control conditions. Again, this prediction was borne out in experiments in which the phase relationships between PY and PD/AB neurons were examined prior to and following IVN stimulation.

These results strongly suggest that asymmetric inputs onto electrically coupled neurons which evoke different responses in a postsynaptic neuron can modulate the phase relationships among these neurons.

J.S. Eisen was a Gillette Fellow at Brandeis University. Research supported by NIH NS17813 and a Sloan Fellowship to E. Marder.

46.8 THE COUPLING OF SEGMENTAL OSCILLATORS CONTROLLING ABDOMINAL PUMPING IN THE LOCUST. Simon F. Giszter* (SPON: R. Lane). Lab. of Dr. G. Hoyle, Inst. of Neuroscience, Dept. of Biology, Univ. of Oregon, Eugene, Oregon.

Many kinds of repetitive animal behaviours appear to be driven by the activity of coupled oscillators. The object of this study is to determine the method of coupling of segmental oscillators driving ventilation in the locust, and how coupling strength varies over a range of cycle periods.

Simultaneous pumping by abdominal segments 2-7 drives a unidirectional airflow through the tracheal system. For efficient airflow segmental movements must be tightly phaselocked. Segmental abdominal ganglia produce appropriately phased antagonistic motoneuron activity in isolation. Hence these ganglia contain a set of coupled oscillators suitable for such analysis.

I have examined segmental interactions in the restrained, resting locust by myographically monitoring specific muscle activity. Sets of records of 250 consecutive pumping cycles were collected for 2 adjacent segments using an on-line PDP11/44 computer. Absolute time of onset, burst duration, interburst duration, and number of spikes per burst were measured and then analysed as time series.

Two parallel pathways transferring information in opposing directions were detected and seem strong enough to account for phaselocking of the segments in the resting animal. Firstly, information moves posteriorly from the metathoracic ganglion to all unfused abdominal ganglia, controlling the onset of inspiration and turning off expiratory activity in all segments. By contrast information controlling the start of expiration travels anteriorly: the intersegmental lag of the expiratory bursts is related to the onset of the next expiratory burst in the more anterior segment by a negative feedforward process. Several neuronal pathways may be contributing to each one of these couplings. Descending interneurons which might be the principle elements in the first coupling have been described (Lewis, Miller & Mill, 1973; Pearson, 1980).

The descending pathway switches expiration to inspiration in all segments, while local control is the more important in phaselocking pairs of oscillators.

Supported by an SRC overseas award and a Morgenroth scholarship to S. Giszter, and NSF Research Grant BNS 82-41884 to Dr. G. Hoyle.

46.9 MOTOR NEURONS AS FUNCTIONAL ELEMENTS OF THE VENTILATORY OSCILLATOR IN THE CRAB. R. A. DiCaprio* and C. R. Fourtner. Dept. of Biological Sciences, SUNY Buffalo, Buffalo, Ny 14260.

In Crustacea, motor neurons as well as interneurons have been shown to play a role in the pattern generator for two sterotyped behaviors, a centrally generated locomotor activity (crayfish swimmeret beating, Heitler, W. J., Nature, 275:231, 1978) and a peripherally generated rhythmic behavior (stomatogastric ganglion, Selverston, A. I., IN Identified Neurons and Behavior in Arthropods, 1977). Simmers and Bush (Brain Res., 197:247, 1980) and DiCaprio and Fourtner (Soc. Neurosci. Abst. 7; 744, 1981) have shown that a number of non-spiking interneurons are elements of the central pattern generator responsible for ventilation in the crab, Carcinus maenas. We report here a more detailed study of the motor neurons associated with the ventilatory system.

Ventilation is the result of rhythmic dorsal/ventral movements of the gill bailer produced by two loosely grouped sets of muscles, the levators and depressors (Young, R. E., J. Comp. Physiol, 101:1, 1975). The motor pattern consists of alternating bursts of the motor neurons to these muscles. Within each of these groups are motor neurons which are active during the initial, middle, and final phases of each burst. Intracellular recordings obtained from motor neurons active in different phases of each group demonstrate that all of these neurons interact with the central pattern generator. Injection of intracellular current pulses into ten ventilatory motor neurons reset the motor pattern, however, the current pulse is only effective when the motor neuron membrane potential is in the depolarizing phase of its oscillation. This resetting effect is not dependent upon spiking activity in the motor neurons, because a current pulse can effectively reset the rhythm when the neuron is held hyperpolarized below its spike threshold. In addition, the injection of constant hyperpolarizing current into motor neurons slows the ventilatory rhythm and in two instances, completely stops the rhythm. Therefore, the motor neurons to the levator and depressor muscles of the gill bailer contribute to the neural circuit responsible for ventilation and this interaction appears to be non-spiking in nature. (Supported by grants 1F32NS06277 to R.A.D.)

46.10 ELECTRICAL COUPLING AND UNCOUPLING OF LEECH SWIM OSCILLATOR NEURONS VIA RECTIFYING ELECTRICAL SYNAPSES. W. Otto Friesen. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Four pairs of segmentally repeated interneurons which serve to generate the leech swimming rhythm have been described previously (Friesen, W.O., Poon, M. and Stent, G.S. J. Exp. Biol. 75: 25-43, 1978). It now appears that this quartet of interneurons is a subset of the complete circuit, for we have discovered four more neuron pairs which also must be considered candidate neurons of the swim oscillator. These additional neurons became evident after we began desheathing the segmental ganglia prior to intracellular recording. The desheathing procedure, which appears not to disturb neuronal function, greatly increases visibility of small neuronal somata and greatly facilitates intracellular recording. We here describe the dual interactions between one of these newly identified interneuron pairs, cells 60, and cell 208, an unpaired oscillator candidate interneuron identified by Weeks (Doctoral dissertation, Univ. of Calif., 1980; Neurosci. Abstr. 7: 137, 1981).

The swim oscillations of the cell 60 pair and cell 208 are antiphasic as a result of the inhibitory synaptic input cell 208 receives from cells 60. This input appears to be direct because: 1) the inhibition is strong, up to 22 mV hyperpolarization per 1.0 nA of current injected into cell 60; 2) the synaptic delay is less than 10 ms and 3) the inhibitory effects persist in Na^+-free Ringers (Tris HCl substituted for NaCl). The inhibitory synapse is effective since hyperpolarization of one cell 60 during swimming activity reduces the hyperpolarizing phase of cell 208 oscillations by about 50%. In quiescent preparations, cells 60 and cell 208 are also coupled via a powerful rectifying electrical connection (coupling strength in the forward direction, cell 60 to cell 208, is about 0.4). The diode connection, if effective during swimming, would act to counter the synaptic inhibition and tend to synchronize cells 60 and cell 208 activities. This antagonistic effect is prevented during swimming activity because cell 208 oscillations, unlike those of cells 60, are superimposed onto a depolarized plateau of about 10 mV, producing a reverse bias across the coupling diode. Thus during swimming activity the current cannot pass through the diode connection, leaving only the synaptic inhibition to set the phase of cell 208. A possible role for the diode interaction could be to synchronize non-swimming activities of cells 60 and cell 208. While such synchronization could also be accomplished via a non-rectifying electrical coupling, the diode connection provides a mechanism for uncoupling these cells during swim activity. Supported by NIH grant NS14965 and NSF grant BNS81-0243.

46.11 THE SWIM-INITIATING ABILITY OF INTERSEGMENTAL SEROTONIN-CONTAINING LEECH INTERNEURONS. M.P. Nusbaum and W.B. Kristan, Jr. Dept. of Biology, UCSD, La Jolla, CA. 92093

Two of the bilaterally-paired serotonin-containing interneurons, designated cell 61 and cell 21, can initiate and maintain the swim motor program in the leech Macrobdella decora. These cells share this swim-initiating ability with cell 204 (Weeks and Kristan, J. exp. Biol. 77: 71-88, 1978) and the serotonin-containing Retzius cells (Willard, J. Neurosci. 1(9): 936-944, 1981). In most respects, this ability is more like that of cell 204 than the Retzius cells in that swim initiation occurs within seconds rather than minutes from the onset of stimulation, and swim initiation by these cells can be effected in large volumes of saline.

Two aspects of the swim-initiating ability of a single cell 61 are different from those of both cell 204 and the Retzius cells. First, the cycle periods it produces are often longer than during swims initiated by cell 204 or the Retzius cells. Second, stimulation of a single cell 61 can sustain the swim motor pattern either in all of the ganglia of a chain, or sometimes exclusively in its own and near neighboring ganglia. Swims initiated by any other means do not result in such a localized generation of the swim motor pattern. However, simultaneous stimulation of two cells 61 causes a swim motor pattern similar to that initiated by these other means.

Cell 61 activity has an excitatory effect on some identified cells in the swim circuit, including: cell 204; the central pattern generating interneuron cell 208; and the dorsal and ventral longitudinal motor neurons. Furthermore, depolarizing cell 204 strongly excites cell 61. During swims initiated by some other means, cell 61 receives rhythmic bouts of excitatory input so that it fires impulses in phase with impulse bursts in cell 208.

Mechanosensory stimulation sufficient to initiate swimming has a strong excitatory effect on cell 61. This activation of T (touch), P (pressure) and/or N (nociceptive) sensory neurons polysynaptically excites cell 61, primarily via the multimodal giant fiber (S cell) system, with which cell 61 has an electrical rectifying connection.

The Retzius cells and cells 61 appear to influence the swimming pattern generator by different mechanisms. The Retzius cells are neurosecretory cells, exerting their effects on the nervous system by increasing the level of serotonin in the blood, whereas the properties of cell 61 suggest that it initiates swimming via discrete synaptic contacts within the ganglion.

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46.12 NEURONS MODULATING HEARTBEAT IN THE LEECH: CENTRAL CONNECTIONS AND PERIPHERAL EFFECTS. R.L. Calabrese and A.R. Maranto (Spon: L.P. Tolbert). The Biological Laboratories, Harvard University, Cambridge, MA 01238.

An elaborate and well-characterized central pattern generator programs the rhythmic beating of the paired hearts in the leech, Hirudo medicinalis. The pattern generator itself comprises a set of segmental heart interneurons. These interneurons rhythmically inhibit a set of segmental heart motor neurons, which time and coordinate the constrictions of the hearts.

We have recently discovered a new class of efferent neurons that receive rhythmic inhibition from the heartbeat central pattern generator. A bilateral pair of these neurons is located in the corresponding position in each of the fifth and sixth segmental ganglia. We have named these cells HA or heart accessory neurons. The HA cells of the fifth ganglion have axons projecting out the contralateral roots of the fifth and more anterior ganglia, while the HA cells of the sixth ganglion have axons projecting out the contralateral roots of the sixth and more posterior ganglia. These peripheral axons extend out to the heart muscle.

Tonic activity in the HA neurons increases the tension produced by the heart muscle in response to heart motor neuron discharge and induces autonomous rhythmic beating of the hearts when heart motor neuron activity is experimentally suppressed.

Experiments are now in progress to determine the electrical effects of the HA neurons on the heart muscle cells.

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46.13 ACTIONS OF ACETYLCHOLINE ON THE RHYTHMIC BURST ACTIVITY OF CARDIAC GANGLION. R. E. Sullivan and M. W. Miller. Bekesy Laboratory of Neurobiology, University of Hawaii, Honolulu, Hawaii 96822.

The cardiac ganglion of Homarus americanus was examined as a possible locus of cholinergic cardioexcitation. Spontaneously bursting isolated ganglia responded to bath application of acetylcholine (10^{-5} - 10^{-3} M) with increases in burst frequency, impulses per burst, and burst duration. Both the motorneurons and premotor cells are excited by acetylcholine. Intracellular recordings from motorneurons revealed 1-5 mV depolarizations which appear to be due to an increase in apparent membrane resistance. Moreover, there was a marked enhancement in the rate of rise of the interburst pacemaker potential.

In the presence of TTX ($3X10^{-7}$ M) bath application of acetylcholine resulted in a concentration-dependent depolarization (K_d = 10^{-4} M) of motorneurons which was effectively antagonized by atropine ($1X10^{-4}$ M). At high concentrations of acetylcholine (10^{-4} - 10^{-3} M) the depolarization was accompanied by repetitive driver potentials. Preliminary manual voltage clamp experiments on isolated motorneurons bathed in TTX saline containing cadmium ($4X10^{-4}$ M) indicated that the depolarization is produced by a net inward current. Similarities between this cholinergic effect and those previously reported for the pentapeptide proctolin (J. Neurobiol. 12, 629, 1981) suggest that isolated cardiac ganglion motorneurons may be a useful preparation for studying the cellular mechanisms underlying peptidergic/muscarinic responses which enhance or induce spontaneity. Supported by NIH grant NS11808 and NSF grant BNS81-07289 to I.M. Cooke and the University of Hawaii Foundation.

46.14 DOPAMINE NEURONS IN THE BUCCAL GANGLIA OF HELISOMA TRIVOLVIS. D. L. Trimble and D. L. Barker. Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

Using the Falck-Hillarp and glyoxylic acid methods, we have located catecholamine-containing neurons on the ventral side of the buccal ganglia of Helisoma. Three populations of catecholamine-containing cells were identified based on cell size and position. Extensive fluorescence was also visible in the peripheral nerve trunks of the buccal ganglia. ^{3}H-Dopamine, but not ^{3}H-norepinephrine, was synthesized from ^{3}H-tyrosine in buccal ganglia. Dopamine synthesis studies of the nerve trunks of the buccal ganglia indicated they all contain dopamine except for the cerebral-buccal connectives, which are the only connections between the buccal ganglia and the rest of the animal's central ganglia. These observations suggest that a dopaminergic system is intrinsic to the buccal ganglia.

The buccal ganglia produce a patterned-motor output (PMO) consisting of alternating bursts of action potentials in retractor and protractor motoneurons. In an attempt to mimick some of the effects that dopamine neurons might have on the PMO, we investigated the effect of bath-applied dopamine on isolated buccal ganglia. Dopamine initiated and/or maintained the PMO of the buccal ganglia, effects similar to those of bath applied 5-HT (Granzow and Kater, Neurosci., 2, 1977). However, the effects of dopamine were more consistent and did not show any tendency to desensitize. The threshold for effects of bath-applied dopamine is about 10^{-7}M, and maximal activation occurs at 10^{-5}M.

In order to demonstrate that the response to dopamine is distinct from the response to 5-HT, haloperidol was used to specifically block the dopamine receptors. Application of 10^{-5}M haloperidol totally blocked the effects of bath-applied dopamine but did not affect the ability of 5-HT to stimulate motor output.

Treatment of the buccal ganglia with haloperidol alone, thereby blocking any spontaneous functioning of dopamine in the system, had several effects on the ongoing PMO of the ganglia. There was an overall reduction in spontaneous activity and there was also a qualitative change in the pattern of the motor output. This is consistent with the hypothesis that at least some of the dopamine neurons in the buccal ganglia normally contribute to the generation of patterned motor output.

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47.1 LOCOMOTION IN THE ADULT CHRONIC SPINAL CAT. S. Rossignol, H. Barbeau and J. Provencher* (SPON: J. Montplaisir). Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

The recovery of locomotor capacities was investigated in four male adult cats (3.9 to 4.4 kg) spinalised at Th 13 under Nembutal anesthesia. The bladder was expressed once a day. The evaluation of treadmill locomotion was started as early as 24 hours after the operation and repeated at least twice a week. Up to now one animal has been tested for 2 weeks, 2 for 3 weeks and 1 for 9 months. In each session, electromyograms (EMGs) were recorded with a pair of copper wires bilaterally inserted into Vastus Lateralis (VL), Semitendinosus (ST) and lumbar Multifidus (Mf). The movements of the animals were simultaneously recorded on videotape.

All cats were capable, within 24 to 48 hours, of small alternate stepping movements at the hip and knee joints although only with perineal stimulation. Thereafter, stepping movements of increasing amplitude were elicited without such stimulation when the dorsum of the feet rubbed against the treadmill belt. Treadmill speeds of up to 1.0 $m.s^{-1}$ could be followed with perineal stimulation but without foot placement or weight support. In one cat bilateral foot placement appeared at the 27^{th} day, at which stage the animal could then, without stimulation, follow speeds of up to 1.4 $m.s^{-1}$. At 8 weeks, this animal could stand on its hindlimbs for more than 3 minutes, with concomitant EMG activity in both VLs. The animal could also walk with complete weight support of the hindquarters for more than 3 minutes at speeds ranging from 0.1 to 0.6 $m.s^{-1}$. These episodes were accompanied by an increase in amplitude of VL activity and the appearance of two bursts per step cycle in each Mf which is typical of axial muscles. The major change occurring thereafter, up to 3 months, was a progressive lengthening of the step duration for the same treadmill speeds. For example, at a speed of 0.2 $m.s^{-1}$, the step cycle duration, measured from consecutive onsets at ST, was 662 ms ± S.D. 87.3 (N=34) at day #12 and 1091 ms ± S.D. 82.4 (N=10) at day #164. This lengthening of the step cycle duration was attributed mainly to an increase in duration of extensor activity but also in part to a longer delay between the end of ST and the onset of VL.

These preliminary results indicate that adult cats progressively recover locomotion capacities after spinalisation, with foot placement and adequate weight support of the hindquarters. (Supported by a Group grant of the Canadian MRC. H.B. received a postdoctoral fellowship from the FRSQ).

47.2 GAIN CHANGES IN CUTANEOUS REFLEXES DURING LOCOMOTION IN THE ADULT CHRONIC SPINAL CAT. C. Julien, H. Barbeau and S. Rossignol. Centre de recherche en sciences neurologiques, Dép. de physiologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

In 8 experimental sessions, stimulation of the skin was applied to the dorsum of the foot during treadmill locomotion (0.3 $m.s^{-1}$) of one adult chronic spinal cat (Th 13). Electromyograms (EMGs) were recorded with a pair of percutaneously inserted copper wires, or with chronically implanted stainless steel wires, from ipsilateral (I/L) and contralateral (C/L) Vastus Lateralis (VL), Semitendinosus (ST), Tibialis Anterior (TA) and Gastrocnemius Lateralis (GL). Mechanical stimulation of the skin was given by contacting the foot with a rod equipped with an electrical switch. Electrical stimulation of the skin (single 5 ms pulse or trains of 10 pulses, 1 msec at 100 Hz, 1-3 mA) was delivered through wires chronically implanted subcutaneously at the dorsum of one foot. Mechanical stimulation during the swing phase induced a short latency (10 to 14 ms) and short duration augmentation of activity of I/L flexors (TA and ST) and the C/L extensor, VL. During stance the same stimulation increased the activity in I/L extensors (VL and GL) as well as in the C/L flexor ST.

Weak electrical stimulation which did not evoke any detectable EMG response during quiet standing was very effective in eliciting large excitatory responses during walking. In 7 experimental sessions, electrical stimuli were given at various phases of the step cycle (defined to start and end at the onset of successive ST bursts). For each muscle, the area of the integrated EMG was measured at a fixed latency (10-12 ms) and a fixed duration (10-20 ms) after the stimulus and an equivalent control period preceding the stimulus was subtracted. During the I/L swing phase, large excitatory responses in I/L ST and TA and C/L VL were found. In the early part of the I/L stance, mainly C/L ST responses were found. For the remainder of the stance phase I/L VL responses were predominant.

After injection of Clonidine, a noradrenergic agonist, the animal walked with longer steps and there was a large increase in the threshold for eliciting the responses, which were, however, essentially similar to those obtained during the control period of walking. Trains of stimuli during standing did not evoke long latency and long duration discharges. It is concluded that during locomotion in the adult chronic spinal cat, there are important tonic and phasic gain changes in the transmission of cutaneous reflexes. Although the threshold for evoking short latency responses may be increased by Clonidine, late discharges generally seen in acute spinal cats injected with noradrenergic drugs have not been seen in the adult chronic spinal cat. (Supported by a Group grant from the Canadian MRC. C.J. supported by an MRC fellowship and H.B. by an FRSQ fellowship).

47.3 MODULATION OF THE LOCOMOTOR PATTERN BY NORADRENERGIC, SEROTONERGIC AND DOPAMINERGIC AGONISTS IN THE ADULT CHRONIC SPINAL CAT. H. Barbeau and S. Rossignol. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

After complete spinalisation at Th13, adult chronic spinal cats are capable of walking on a treadmill with foot placement of the hindlimbs and weight support of the hindquarters. In the present study, we investigated the effects of monoamine agonists on the locomotion of a cat chronically spinalised for more than 60 days and in which all descending monoaminergic terminals are presumed to be degenerated. In 7 experimental sessions, the electromyographic (EMG) activity of Vastus Lateralis (VL) and Semitendinosus (ST) was recorded bilaterally through percutaneously implanted copper wires. The movements of the animal were also simultaneously video-recorded before and after the injection of drugs.

Clonidine (150 µg/kg), a noradrenergic agonist, markedly modified the locomotor pattern, especially at low speeds (0.05-0.6 $m.s^{-1}$). There was an increase in the step length, a better foot placement, an elevation of the hip indicating a better weight support and a pronounced abduction of the hindlimbs. For example, at a speed of 0.2 $m.s^{-1}$, the control cycle duration was 959 ± 32 ms (N= 13) while it increased to 1984 ± 69 ms (N= 10) after Clonidine. The ST and VL bursts were at least twice as long while the amplitude remained similar to those of the control period. With Quipazine (1 mg/kg), a serotonergic agonist, there was an increase of extensor tonus at rest. During walking, the hips were markedly raised and the hindlimbs tended to adduct. The cycle was also prolonged at 0.2 $m.s^{-1}$ from 910 ms ± 67 ms to 1412 ms ± 77 ms (N= 7). The ST bursts duration remained unchanged. The VL bursts only slightly increased in duration but at least doubled their amplitude. Apomorphine (0.5 mg/kg), a dopaminergic agonist, produced a marked tendency for a sustained flexion of the hindlimbs which could disrupt the locomotor pattern. The amplitude of ST bursts was increased and that of VL was decreased. The cycle length was virtually the same at 0.2 $m.s^{-1}$ as that of control.

Previous observations indicated that a well developed locomotion is possible in adult chronic spinal cats in the absence of bulbo-spinal monoaminergic endings. However, the present results show that the stimulation of aminergic receptors modifies locomotion significantly, suggesting that the activation of these receptors may normally serve to modulate the overall expression of the spinal locomotor pattern generator by changing, for instance, the duration or the amplitude of the EMG activity. (Supported by a Group grant from the Canadian MRC. H.B. received a postdoctoral fellowship from the FRSQ).

47.4 MICROSTIMULATION OF THE MEDULLARY RETICULAR FORMATION AT REST AND DURING WALKING IN THALAMIC AND INTACT CATS. T. Drew*, R. Dubuc and S. Rossignol. Centre de recherche en Sciences Neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

Stimulation of the medullary reticular formation (MRF) during locomotion in high decerebrate and thalamic cats was reported to augment flexor burst activity and to diminish the extensor burst in the ipsilateral (I/L) hindlimb depending on the time of occurrence of the stimulation in the step cycle (Orlovsky, G.N., 1972, Brain Res. 40: 359). Experiments were designed to study the responses to MRF stimulation (11 pulses, 0.2 msecs, 330 Hz, $\leq$35 µA) in thalamic and chronically prepared cats (7 and 2 animals respectively) at rest and during walking on a moving belt.

Glass-coated tungsten microelectrodes (2-5 MΩ) were stereotaxically inserted into the MRF at coordinates in the range of AP -6 to -12, L 0.5 - 2.5. Stimuli were delivered throughout tracks where field potentials as well as single unit firing could be antidromically evoked by stimuli delivered to the spinal cord (L_1) by implanted microwires. Electromyograms (EMGs) from up to 14 muscles were recorded in the neck, all four limbs and the back. The results from both preparations were essentially similar and will be presented together.

At rest the predominant response in the forelimbs was an I/L elbow flexion and a contralateral (C/L) elbow extension although bilateral elbow extension was also seen at some stereotaxic coordinates of the MRF. It should also be noted that EMG was often evoked in the antagonist muscles to those causing movements. In the neck, bilaterally evoked responses were most common although head movement was always to the I/L side. The largest forelimb responses were normally from the location at which the maximal antidromic field potential was evoked and at this position activity was also normally evoked both in the I/L Quadriceps group and bilaterally in the back muscles. Latencies of the earliest effects in the forelimbs were of the range 9-15 msecs and for the neck 6-10 msecs.

During locomotion the patterns of muscular activity evoked by the MRF stimulation depended critically on the phase of the step cycle during which the stimulation was applied. Stimulation applied during the I/L forelimb stance period often evoked strong activity in both I/L forelimb and hindlimb extensors with concomitant flexion of the C/L flexor muscles. Stimuli given during the swing phase of the I/L forelimb caused hyperflexion of I/L fore- and hindlimbs with extensor responses on the C/L side. It is concluded that the MRF is capable of effecting changes in the pattern of activity in flexors and extensors of all four limbs. (Supported by a Group Grant of the Canadian MRC; T. Drew supported by a Canadian MRC Postdoctoral Fellowship).

47.5 TESTING THE ROLE OF RENSHAW CELL RHYTHMICITY DURING FICTIVE LOCOMOTION. J. Jamal*, B. Noga*, S.J. Shefchyk* and L.M. Jordan (SPON: R.M. Jell), Department of Physiology, University of Manitoba, Winnipeg, Canada.

Renshaw cells (RCs) are known to be excited by motor axon collaterals and to produce inhibition of several types of spinal cord neurons, including alpha and gamma motoneurons and Ia inhibitory interneurons, suggesting a role for RCs in the shaping of motor output. Although RCs have been demonstrated to be rhythmically active during locomotion (McCrea et al., J. Neurophys., 44:475, 1981), their contribution to this motor activity is unclear. It has been demonstrated that the excitation of RCs is due in part to the action of acetylcholine at nicotinic receptors (Curtis and Ryall, Exp. Brain Res., 2:66, 1966). Furthermore, the intravenous administration of the nicotinic antagonist mecamylamine (MEC) has been shown to effectively antagonize the excitation of RCs produced by antidromic stimulation of motoneuron axons (Ueki et al., Exp. Neurol., 3:141, 1961). The present research examines the effect of MEC on RC and motoneuron activity during fictive locomotion.

Locomotion was induced by stimulation of the mesencephalic locomotor region in mesencephalic preparations (as described by Jordan et al., Brain Res., 177:204, 1979). The animals were paralyzed using gallamine triethiodide, which has no effect on RC discharge (Brooks and Wilson, J. Physiol., 146:380, 1959).

RC spiking and recurrent IPSPs in motoneurons produced by electrical stimulation of a cut ventral root were greatly diminished following i.v. doses of 1-3 mg/kg MEC. While all of the RCs examined displayed rhythmic activity linked to the locomotor cycle prior to drug administration, RC rhythmic activity during fictive locomotion was completely abolished by the drug. At the same time, the pattern of fictive locomotion monitored from peripheral motoneuron axons was unchanged. The rhythmic bursting activity of motoneurons was altered only to the extent that the number of spikes per burst was increased. No significant changes in the rhythmic membrane potential oscillations of motoneurons recorded intracellularly were observed, but previously undetected EPSPs were revealed during the depolarized phase of the step cycle.

These results show that RC rhythmicity during locomotion is determined by excitatory drive from motor axon collaterals. RCs appear to limit motoneuron spiking and to control excitatory input to motoneurons, but they do not contribute significantly to the factors determining the membrane potential oscillations in motoneurons during fictive locomotion.

Supported by a grant from the Medical Research Council of Canada (MRC) to LMJ. SJS is a predoctoral student of the MRC.

47.6 *TEMPORAL COORDINATION OF TWO COINCIDENT MOTOR PROGRAMS: LOCOMOTION AND HINDLIMB PAW SHAKE.* *M.C. Carter*, C.A. Giuliani* and J.L. Smith (SPON: C.M. Harris) Dept. of Kinesiology and Brain Research Institute, UCLA, CA 90024.*

It has been hypothesized that locomotion (Grillner, et al. Exp. Brain Res. 34:1979) and the paw shake response (Smith, et al. Neurosc. Abst. 1980) are rhythmical behaviors organized primarily by central pattern generators in the spinal cord. However, these centrally programmed movements differ with respect to their patterns of motoneuron recruitment and cycle times. Thus, production of locomotion and paw shaking concurrently may provide insight as to how the CNS coordinates two distinct motor programs.

Two normal cats were chronically implanted with bipolar electrode wires in the right and left lateral triceps brachii (RLT, LLT) and soleus (RSO, LSO) as well as the right tibialis anterior (RTA) and lateral gastrocnemius (RLG). EMG records obtained during normal locomotion at 0.7 m/s were compared with records from walking and concurrent shaking at the same treadmill speed. Paw shaking was induced by applying tape to the plantar surface of the right hindpaw. Cycle time (CT, measured from successive LSO burst onsets), burst durations and onset latencies normalized to percent of CT, were obtained from the EMG records.

The cats did not shake the taped hindpaw during each step cycle, rather the response was intermittent and confined to the swing phase. Analyses were limited to cycles in which the cat locomoted at a relatively fixed point on the treadmill. During normal locomotion average CT was 638ms ± 40. The SO and LT muscle pairs were active on average 50% of the cycle, while the RTA was active for 20%. Activity of the RLG was more variable and ranged from 30-40% of the cycle. Onset latencies for all muscles were very consistent: RSO and RLG occurred at 50% into the cycle (50% ± 4 and 55% ± 3), while the RTA and RLT onsets were within the first 20% (20% ± 2 and 19% ± 6). Onset of the LLT occurred later in the cycle and averaged 73% ± 5. Comparisons between normal locomotion and cycles where the shake occurred revealed the step cycle was modified in one of two ways. In some instances CT increased 150ms on the average, yet normalized burst durations and onset latencies remained constant. Thus, the entire cycle lengthened proportionally. On other trials CT did not change, but LSO and RTA burst durations increased as RSO decreased, indicating the stance phase of the taped hindlimb shortened while the swing phase increased as the paw completed 2-3 shake cycles. Forelimb coordination appears unaffected by paw shaking.

Our data suggests that the two motor programs are temporally coordinated such that there is efficient time-sharing of the motoneuronal pools. Muscular phasing is modulated in such a way as to accommodate brief hindpaw shaking while maintaining continued locomotion. Supported by NIH Grant, NS 16333.

47.7 *AIRSTEPPING IN THE CHRONIC CORDOTOMIZED CAT.* *C.A. Giuliani*, C. Sabin* and J.L. Smith (SPON: E.E. Decima). Dept. Kinesiology and Brain Research Institute, UCLA, CA 90024.*

Airstepping has been identified as rhythmic alternating movements of the hindlimbs that commonly occurs in chronically cordotomized cats when they are suspended vertically with hindlimbs pendant (Sherrington, J. Physiol. 40:28, 1910). Evidence from Grillner's lab (Brain Res. 43:24, 1979) supports the concept of a spinal central pattern generator capable of producing locomotor-like rhythms which may resemble airstepping (ASTP). The present study attempted to identify the temporal characteristics of ASTP recorded in chronic cordotomized cats as compared with the characteristics observed in treadmill and fictive locomotion.

Data were obtained from 4 cats: 2 were cordotomized at 8 mos of age, 1 each at 2 wks and 3 mos of age. ASTP was evaluated at 1-3 mos after cordotomy. The soleus (SO), lateral gastrocnemius (LG), tibialis anterior (TA) and vastus lateralis (VL) of left hindlimbs were chronically implanted with bipolar electrode wires. EMG recordings were synchronized with videotaped movements of spontaneous ASTP as well as ASTP during gentle pinching at the base of the tail (TP). EMG parameters analyzed included: cycle time (CT, defined as the interval between SO burst onsets), latency of burst onset normalized to percent of CT for evaluation of intralimb coordination, and burst duration.

A total of 87 cycles of spontaneous ASTP were analyzed. The average CT, 533 ±107 ms was much shorter than CT recorded during weight-supported treadmill locomotion that ranged on average from 1.25s at 0.2m/s to 600ms at 1.2m/s in cordotomized cats (Smith, J.L., Exp. Neurol., 1982, in press). Intralimb coordination during ASTP was similar to that seen during treadmill locomotion of both cordotomized and intact cats. In 90% of the cycles studied, burst onset of the extensor synergists, SO/LG and SO/VL, occurred within the first 10% of the cycle, while the TA burst onset averaged 78% ±8 into the cycle. Of 121 cycles analyzed, TP altered ASTP in each cat. Variability of CT increased, while the average CT was unchanged in 3 cats and markedly increased in 1 cat. During TP, intralimb coordination of the extensors was unaffected, although an increased variability of TA onset latency was observed. The influence of TP on SO and TA burst duration varied in each animal and overall burst duration was more affected by TP than was CT.

Our data suggest that the intralimb coordination during ASTP is similar to that seen in treadmill locomotion of chronic cordotomized or intact cats, as well as to the rhythms seen during fictive locomotion. Thus, ASTP may provide a good behavioral model in which to examine activity produced by the central pattern generators in the lumbosacral cord.
Supported by NIH grant NS 16333.

47.8 RECOVERY, RECEPTIVITY, AND PERTURBATION OF PAW SHAKING IN CHRONIC SPINALIZED CATS. C. Sabin*, C.A. Giuliani* and J.L. Smith. Dept. of Kinesiology and Brain Research Institute, UCLA, CA., 90024.

Previous studies have indicated that paw shaking responses (PSR), rapid alternating flexion and extension of the hindlimb to a cutaneous stimulus, can be elicited in spinalized cats, with temporal characteristics resembling those of intact cats (Smith, Neurosci. Abst., 1980). The purpose of the present study is to describe the recovery of PSR following cordotomy as well as to determine the receptivity of the PSR to a wide range of stimuli. In addition, PSR was studied with afferent perturbations imposed by immobilization.

Five female cats were cordotomized at T_{12} as young adults (8 mos or older) and bipolar fine wire electrodes were surgically implanted into the lateral gastrocnemius (LG). PSR was elicited by applying masking tape to the hindpaw at the level of the central plantar pad. To determine the temporal characteristics of PSR, cycle times (CT), defined as the time between two consecutive LG burst onsets, were measured. Recovery of PSR was evaluated from EMG recordings taken 2x weekly for 40 days after cordotomy. Receptivity of PSR to a range of cutaneous stimuli was evaluated using water (25°C), ice, hot (50-70°C), and cold (0-15°C) stimuli in addition to tape. Hot and cold stimuli were applied using a metal plate, affixed to a force transducer, which could be heated or cooled. In some cats PSR was tested subsequent to immobilization of the ankle (110° flexion) and knee (115° flexion) in a plaster cast.

Paw shaking responses were observed as early as 48 hours after cordotomy. From days 2-10 after cordotomy, CT ranged from 105 to 109 ms. From days 11-20, the CT decreased to normal values of 80-94 ms and was stable throughout the remaining testing period (21-40 days). Receptivity to varying stimuli was evaluated according to consistency of PSR elicitation with each stimulus as well as by counting the number of cycles within a PSR. Tape and water were the most effective stimuli, eliciting PSR in 76% of the trials. Ice, hot, and cold stimuli tended to elicit flexion withdrawl in a majority of the trials. In addition, tape elicited PSR averaged 5 cycles longer than responses elicited by other cutaneous stimuli. Immobilization produced an increase in CT from an average of 96 ms to 134 ms and on 16% of the trials, the LG exhibited abnormal, lower amplitude EMG bursts that occurred between the normally fast-rising, short-duration PSR bursts.

Our results suggest that paw shaking is an automatic movement triggered by a variety of cutaneous cues that is coordinated by central pattern generators in the lumbosacral cord. Lack of normal phasic feedback does not disrupt the coordination, but lengthens the CT.

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47.9 LOCOMOTION INDUCED BY INTRATHECAL DRUG ADMINISTRATION IN SPINAL CATS. D.J. Omeniuk* and L.M. Jordan (SPON: K.W. Cheng), Dept. of Physiology, Univ. of Manitoba, Winnipeg, Canada.

Available evidence suggests that locomotion is patterned at the level of the spinal cord, and that signals from the brainstem are normally required for the activation of the central pattern generator for locomotion. Norepinephrine (NE) and dopamine (DA) have been implicated as transmitters in pathways descending from the brainstem to the spinal cord, and L-DOPA and clonidine (an agonist of NE) can induce locomotion in spinal animals (see Grillner, Physiol. Rev. 55:247, 1976). In contrast, locomotion induced by brainstem stimulation is not abolished by depletion of spinal and brainstem NE (Steeves et al., Brain Res. 185:349, 1980). Commissiong has suggested DA as the mediator of L-DOPA effects in the spinal cord (Fed. Proc. 40:2771, 1981). This study was conducted in order to assess NE and DA as possible transmitters involved in the initiation of locomotion, and to determine the feasibility of intrathecal drug injection as a means for initiation of locomotion in spinal animals.

Cats (n=18) were spinalized in the lower thoracic region by cutting the cord with scissors (n=11) or by crushing the cord with forceps (n=7). A cannula was inserted into the subarachnoid space so that the tip reached the lumbosacral enlargement. The animals were rated on the basis of their ability for weight bearing (WB) and generation of rhythmic locomotor movement (RM). The cats were also filmed as they walked on a moving treadmill belt, and the kinematics of the gait were analysed. Drugs used were NE and glutamic acid (Glu), which were dissolved in Elliots Solution A, and DA dissolved in saline with ascorbic acid (10 mg ascorbate/50 ml saline). Control injections were done with the vehicle solutions.

NE elicited increased WB and/or RM in 13 of 14 cats. Concentrations of 10^{-4}M or greater were effective. The quality of RM and WB obtained were sufficient to propel the cats along a treadmill or overground with a support for lateral stability. Once locomotion was induced by NE, subsequent doses were more effective. DA was considerable less effective than NE in evoking locomotion, especially when administered after a time period long enough for degeneration of noradrenergic terminals (7 to 9 days post-transection). Glu could induce RM but not WB.

This study establishes the feasibility of locomotor induction in spinal animals by intrathecal drug administration, and it demonstrates that walking with weight support can be achieved in adult spinal animals. The fact that DA effects were comparatively slight suggests that DA plays little role in the production of spinal locomotion by L-DOPA. The results with Glu show that the NE effects are not likely to be due simply to global excitation.

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47.10 MESENCEPHALIC LOCOMOTOR REGION (MLR) UNIT ACTIVITY DURING LOCOMOTION. E. Garcia-Rill, R. D. Skinner and J. A. Fitzgerald*. Dept. Anatomy, Univ. Arkansas for Med. Sciences, Little Rock, AR 72205.

The MLR is an area of the cat posterior mesencephalon which when stimulated induces locomotion as long as (a) a precollicular-postmamillary transection has been performed and (b) the animal's weight is supported by a hammock. Stimulation of the MLR is not necessary, however, if a precollicular-premamillary transection is performed, since there is spontaneous locomotion. The activity of single neurons in the area of the MLR was studied in the spontaneous locomotion preparation. Under short-acting barbiturate anesthesia the transection was performed and a recording well implanted over the MLR following suction ablation of the overlying cortex and extirpation of the tentorium. Glass micropipettes were advanced by a miniature microdrive supported by the well and the location of recorded neurons was marked by deposition of Fast Green dye. EMG's were recorded from flexors and extensors of each limb.

The spontaneous locomotion preparation is characterized by episodes of four-, two- and one-limb walking mixed with periods of no activity. This allowed assessment of the relationship between unit activity and EMG(s). A total of 55 units which were localized within the MLR were studied from 5-45 min. These cells were located in the cuneiform nucleus and in the nucleus tegmenti pedunculopontinus, which is embedded in the brachium conjunctivum. Close to 30% of these units fired rhythmically in relation to the alternating EMG patterns. Analysis of firing patterns determined that six units fired in advance of contralateral forelimb flexor EMG. A similar number of units fired in relation to the EMG from both forelimbs or both hindlimbs. Other units fired in relation to a complete two- or four-limb step cycle.

In addition, a total of eleven single cells were studied in the Interstitial nucleus of Cajal (INC). Four of these units were found to fire in relation to two-limb or four-limb EMG patterns. Projections of the INC are described elsewhere at this meeting.

Our results suggest that neurons in the MLR and, perhaps, the INC may ultimately influence the firing of locomotion oscillators at the level of the spinal cord.

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47.11 EFFECTS OF COMPLETE UNILATERAL HINDLIMB DEAFFERENTATION ON LOCOMOTION IN CATS. C. S. Lewis*, L. C. Maxwell* and E.Eidelberg Research Program, Veterans Administration Hospital, and U. of Texas Center for the Health Sciences, San Antonio, TX. 78284

Cats (2-4k_g) were trained to walk on a moving treadmill at speeds of 0.4 to 0.6 meters/second. Then the left hindlimb was deafferented by intradural section of dorsal roots L_1 to S_1. The extent of the deafferentation was verified by serial histological sections, which also served to look for inadvertent damage to the spinal cord. The findings described below were obtained in cats where the deafferentation was complete and there was no histologically significant cord injury.

The pattern of locomotor activity of the deafferented hindlimb was studied by conventional kinematic methods from movie films taken prior to the surgery and serially after it for 6 weeks. Muscle samples were taken from extensor digitorum longus, soleus and vastus lateralis, quick frozen and subjected to routine histochemical analysis. Fiber type distribution and fiber cross-sectional areas were examined in the deafferented limb, the intact contralateral limb and in both limbs of sham-operated controls.

Severe and persistent deficits in stepping were found. Some recovery was observed within the first two weeks and stabilization had become clearly apparent by 4 weeks. The deficits included altered rhythmicity, loss of coordination, and reduced amplitude of the hip flexion. The knee and ankle joints were hyperextended and their movements paralleled those of the hip. This pattern differs significantly from that previously described by others. We conclude that sensory feedback is essential for normal locomotor function.

47.12 HOW IS THE MONOSYNAPTIC REFLEX MODULATED DURING THE STEP CYCLE? S. J. Shefchyk, R. B. Stein and L. M. Jordan, Depts. of Physiology, University of Manitoba, Winnipeg, Canada, and University of Alberta, Edmonton, Canada.

If brief stretches are applied to a muscle of a high decerebrate cat which is induced to walk by stimulation of the mesencephalic locomotor region, a pronounced modulation of the monosynaptic reflex response is observed during each step cycle (Aldridge et al. Soc. Neurosci. Abstr. 7:560, 1981). Electrical stimulation of nerves at a strength which excites only the largest afferent fibers produces a similar modulation of the H-reflex. This modulation could be produced either by cyclic variations in the monosynaptic EPSP evoked by homonymous motoneurons or by cyclic variations in the membrane potential (Em) which would vary the probability of the EPSP's producing nerve impulses.

To examine these issues, intracellular recordings from identified motoneurons were made during fictive locomotion in the mesencephalic preparation (as described by Jordan et al. Brain Res., 177:204, 1979). Data were obtained for the following motoneuron types: triceps surae, tibialis anterior, semitendinosus and posterior biceps. Conductance was measured using current injections of short hyperpolarizing pulses through the recording microelectrode (as described by Shefchyk et al. Soc. Neurosci. Abstr. 7:687, 1981). No significant changes in motoneuronal conductance were observed during the step cycle. Therefore, conductance changes could not account for any changes observed in the monosynaptic EPSPs. In some motoneurons (47% of cells sampled) the monosynaptic EPSP displayed small cyclic variations with the maximum amplitude occurring during the depolarized phase of Em oscillations during the step cycle ("in phase" modulation), as suggested by Schomburg and Behrends (Brain Res., 143:533, 1978). In other motoneurons the peak EPSP amplitude occurred during the Em hyperpolarization (12%) or displayed no modulation at all (41%). For the entire population examined, no significant trend was observed, although there was a tendency for "in phase" modulation to occur in those cells with the greatest variation of the Em during the step cycle. Thus, the previously observed modulation of the monosynaptic reflex during locomotion appears to occur mainly by Em variation, although some motoneurons also show EPSP modulation.

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47.13 THEORY OF LATERAL LINE SYSTEM FUNCTION IN TWO DIMENSIONS
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A simple theory of lateral line system function is proposed, based on the known anatomy and physiology of this system in the shark. The lateral line receptors are found bilaterally within the dermis, running the length of the body. Individual lateral line receptors are very sensitive to low frequency vibrations in the surrounding water, and are able to provide a neural signal in the lateral line branches of the cranial nerves after transducing this mechanical disturbance. Phase differences in contralateral lateral line nerves would thus correspond to the phase difference in the wave detected by the contralateral lateral line detectors (Schuijf,1981). This phase information is fed via the lateral line nerves to granual cell clusters and hence the parallel fibers which run transversely across the lateral line lobe (Larsell,1967). If, as has already been suggested (Braitenberg,1967), the slowly conducting parallel fibers of the cerebellum act as timing devices, then the phase differences between the wave fronts at contralateral lateral line receptors can be measured in terms of the distance the neural signal can travel from the midline before meeting its counterpart traveling in the opposite direction along an adjacent parallel fiber towards the midline. Intersection of activity on one side rather than the other would represent a lag in the phase of the wave activating the lateral line receptors on that side. The parallel fiber region in which the two signals meet would provide a maximum of activation of the Purkinje cells in that region. Such activation could be used to modify the direction of swimming.

Such unbalanced activity in the lateral line lobe could account for the ability of the shark to home in on the source of vibration. Purkinje cells with dendrites intersecting the parallel fibers in a region of maximum excitation would be activated and would then inhibit motor neuron activity on the same side. Reduction in motor neuron activity on one side would produce a lessened contraction of the trunk swimming wave on that side, so as to produce a turn in the swimming. The turn would continue until the neural activity , and hence muscular activity, is balanced, in which case the shark would be heading directly towards the source.

Since two lateral lines run one above the other on each side of the head region, phase determinations are in principle possible in the yaw-axis allowing interception in three-dimensions. Further since the vestibular apparatus is thought to have evolved from the lateral line detectors, the vestibular cerebellum of all vertebrates including that of humans, may function in a similar way.

47.14 EFFECT OF CHRONIC SOMATOSENSORY DEAFFERENTATION AND DARKNESS ON SELF-DIRECTED BEHAVIORS AND THEIR RELATION TO SELF-INJURY IN MONKEYS.
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The behavior of seven monkeys (Macaca fascicularis) with chronic, unilateral forelimb deafferentation (4-5 years postoperative) and four normal monkeys were recorded on videotape. The frequency of several categories of self-directed behaviors to different parts of the body was tabulated. 1. The incidence of self-grooming acts was no different for the deafferented limb than for the contralateral, intact limb or the corresponding limb in normal animals. Thus, the stimulus for the control of grooming may not be somatosensory in nature. 2. Scratching to the deafferented limb was greatly reduced, but was not abolished. The scratching that persisted was directed predominantly to the deafferented shoulder, which bordered intact portions of the body. Scratching to the intact, contralateral forelimb in deafferented animals was elevated. 3. More nibbling and licking was directed toward the deafferented arm than to the intact arm. This difference was completely accounted for by animals with lesions on their arms. 4. Mouth capture, defined as taking a part of the body between the teeth without breaking the skin, was observed much more frequently in deafferented than in normal limbs, usually directed toward the area of a lesion. This behavior was essentially passive in nature; it was never observed to have an aggressive character. 5. Biting, defined as an act in which the skin was penetrated, was not observed either to intact or deafferented limbs.

In order to test whether self-grooming is under the control of vision, five of the monkeys were kept continuously in total darkness. The monkeys exhibited a greatly reduced amount of self-directed behaviors, most markedly grooming (reduced to 4%), but also licking (14%), nibbling (27%) and scratching (58%). This result cannot be explained by a reduction in the general activity level in the dark; it suggests that in monkeys the performance of these self-directed behaviors, especially grooming, is under visual control.

Results suggest that maintenance and enlargement of lesions in chronically deafferented monkeys are due to an elevation in behaviors that are passive in character (licking, nibbling), and not to aggressive or other acts (scratching, biting) that might occur in response to dysesthesias. Moreover, darkness which should not affect dysesthesias, reduced all self-directed behaviors. The absence of dysesthesias is consistent with the human deafferentation literature. The question of whether dysesthesias are present in some animals, including monkeys, for an initial period following dorsal rhizotomy still requires resolution.

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47.15 THE EFFECTS OF REEFFERENT AND REAFFERENT FEEDBACK ON MOVEMENTS OPPOSING ELASTIC FORCES.
D.E. Teoduru, T.A. Tran* and A.J. Berman. Dept. of Neurosurgery, VA Medical Center, Bronx, NY 10468.

Reefferent loops (collateral discharge) provide a "sense of effort" (McClosky, 1981) while reafferent loops (proprioceptive feedback) provide information on the status of muscles and joints (Evarts, 1981). This study sought to examine the effects of reefferent and reafferent inputs on limb movements exerted against an elastic force. In a trace-avoidance conditioning paradigm, at the sound of a click, intact, unilateral (uDR) and bilateral (bDR) dorsal rhizotomized (C_2 to T_3) monkeys were required to exert a criterion force (CF), flexing an unseen forelimb to stretch a spring a required distance (each 1/2 in. = 1 lb. of force). The first peak (PF), the first peak to reach criterion (PC) and the highest peak (PH) were recorded in lbs. of force on all trials.

Intact monkeys exhibited an ascending saw-tooth shaped response where PF<PC<PH; thus PC was generally within a wide envelope formed by PF and PH. uDR monkeys showed a narrower PF-PH envelope where PC was closer to CF than the PC of intact and uDR monkeys. Whenever a monkey avoided shock, on 80% of the trials on two consecutive days, CF was increased by 1/2 lb. increments. uDR and bDR monkeys were able to change their movements so as to overcome the spacial restraints imposed on the limb by the apparatus. An appropriate response seemed to be systematically hunted for and when found was performed with the DR limb in a stereotype manner, suggesting that new motor programs can be developed in open loop fashion.

These results suggest that reefferent feedback is adequate to guide open loop extensions of force. Intact monkeys perform rapid unopposed limb movements in open loop fashion (Fromm and Evarts, 1978) as ascending afferents are blocked by corticofugal motor collaterals (Felix and Weisendanger, 1971). However, when large forces oppose movements, reafferent inputs may overwhelm these inhibitory systems, interferring with open loop execution of the movement and making it discontinuous (Brooks, 1979). In uDR monkeys, reafferents from the intact side may have a smaller but similar effect on movements of the DR limb. Since PH of intact monkeys exceeded CF much more than the PH of DR monkeys, we conclude that reafferent inputs interfere with reefferent readouts of effort.

47.16 PHYSOSTIGMINE: ANTAGONISM OF ANTIMUSCARINIC INDUCED DISRUPTION OF EQUILIBRIUM PERFORMANCE IN MACAQUES,
C. T. Bennett, D. N. Farrer*, and N. L. Lof*. School of Aerospace Medicine, Brooks Air Force Base Texas 78235.

Nine macaques were trained to perform a compensatory tracking task that required them, while in a restraining chair, to maintain a relatively vertical position. Adjusted root mean square (ARMS) of the platform movement was the primary dependent variable. In the first of three studies, the monkeys were injected with saline or 0.05, 0.10, or 0.15 mg/kg (IM) of physostigmine. A significant disruption in equilibrium performance was observed to occur within 15 minutes. The animals behavior returned to within normal limits in 90 minutes. In the second study, animals were injected with 0.25 mg/kg (IM) of atropine, then, 90 minutes later with saline or 0.05, 0.10, or 0.15 mg/kg (IM) of physostigmine. The latter injection was timed to coincide with the maximum affect of atropine. Significant antagonism of the antimuscarinic affect on equilibrium was observed to occur within 15 minutes. In the third study, the same series of doses were used, but injections were administered at the same time. Not only did we observe an antagonism of the early affects of physostigmine, which occur prior to the behavioral actions of atropine, but, also, reversal of the antimuscarinic affects, which occur after the behavioral affects of physostigmine have subsided.

47.17 PROJECTIONS OF THE INTERSTITIAL NUCLEUS OF CAJAL (INC) TO THE AREA OF PROBST'S TRACT. 1. ANATOMY. S. Griffin*, R. Nelson*, R. D. Skinner and E. Garcia-Rill (SPON: T. Sims). Dept. Anatomy, Univ. Arkansas for Med. Sciences, Little Rock, AR 72205.

The Mesencephalic Locomotor Region (MLR) of the cat is known to send downstream projections in the area of Probst's tract (Anat. Rec. 199, 90A, 1981). Injections of fluorescent dye into the area of Probst's tract retrogradely label cells in the MLR. Elsewhere at this meeting we report that the activity of some MLR neurons is related to the EMG pattern produced by locomotion. In addition, a small number of INC cells showed similar characteristics. Since stimulation of the area of Probst's tract (Pontobulbar Locomotor Strip) is known to induce locomotion, it became important to determine if descending projections of the INC travel in the area of Probst's tract.

Under barbiturate anesthesia, fluorescent dyes (DAPI or Bisbenzimide) were injected (0.05 µl) into the area of Probst's tract, or the medial longitudinal fasciculus (MLF). Following appropriate survival times, the animals were anesthetized and perfused transcardially with buffered formalin. The brains were treated according to standard procedures for fluorescence microscopy. Following injections of dyes into the MLF, retrogradely labeled cells were observed bilaterally in the INC, although in greater numbers ipsilaterally. Following injections of dyes into the area of Probst's tract, many retrogradely labeled cells were seen in the contralateral INC, and only a few cells ipsilaterally. In cases in which dye injections spread into the medial vestibular nucleus, a greater number of ipsilateral INC cells were labeled without contralateral INC labeling.

In addition to contralateral INC labeling, injections in the area of Probst's tract induced retrograde labeling in the mesencephalic trigeminal root, cuneiform nucleus, nucleus tegmenti pedunculopontinus, and medial central gray. Very sparse labeling was observed in the substantia nigra, subthalamic and entopeduncular nuclei.

Our results suggest that, in addition to the known descending projections of the INC to the ipsilateral medial vestibular nucleus and ipsilateral MLF, a primarily contralateral projection descending in the area of Probst's tract is present. Coupled with evidence showing rhythmic activity in INC during locomotion and induced locomotion following stimulation of the area of Probst's tract, these data suggest INC may also be involved in the modulation of locomotion oscillators at the spinal cord level.

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47.18 PROJECTIONS OF THE INTERSTITIAL NUCLEUS OF CAJAL (INC) TO THE AREA OF PROBST'S TRACT. 2. ELECTROPHYSIOLOGY. R. D. Skinner, J. A. Fitzgerald* and E. Garcia-Rill. Dept. Anatomy, Univ. Arkansas for Med. Sciences, Little Rock, AR 72205.

Injections of fluorescent dyes into the area of the medial longitudinal fasciculus (MLF) in the cat produce retrograde labeling primarily in the ipsilateral INC, whereas injections into the area of Probst's tract produce labeling in the contralateral INC (see accompanying presentation). Electrophysiological experiments were undertaken to confirm these anatomical observations. Extracellular recordings using glass micropipettes were made in the INC. Responses were studied following stimulation through comb electrodes spanning the medulla from the MLF medially to the area of Probst's tract dorsolaterally. Surgical procedures were carried out under barbiturate anesthesia and recordings under locally anesthetized, paralyzed conditions. Fast Green dye was deposited at various recording sites. A total of 131 neurons were identified within the boundaries of the INC. Forty-seven could be antidromically activated from the medulla (or from comb electrodes at C1). Close to 50% of these INC neurons responded antidromically to MLF stimulation. About 20% responded antidromically to contralateral Probst's tract stimulation, and an additional 20% responded antidromically to both MLF and contralateral Probst's stimulation. Close to 10% were activated antidromically from the MLF and ipsilateral Probst's tract stimulation. The latency for antidromic responses following MLF stimulation at medullary levels was 1.0 ± 0.5 S.E. mean msec, and 1.4 ± 0.7 S.E. mean msec following MLF stimulation at C1 levels. The mean latency for antidromic responses following Probst's tract stimulation at medullary levels was 1.2 ± 0.5 S.E. mean msec.

Orthodromic responses were observed in 13 of the antidromically responsive neurons and in 21 otherwise non-responsive units at latencies ranging from 2-30 msec.

Our results confirm the presence of a significant descending projection from the INC to the contralateral Probst's tract region, in addition to the known ipsilateral descending projections to MLF and medial vestibular nucleus.

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47.19 AFFERENT CONNECTIONS OF THE VENTRAL TEGMENTAL NUCLEUS OF GUDDEN IN THE CAT AS REVEALED BY HORSERADISH PEROXIDASE GEL IMPLANTS AND TETRAMETHYLBENZIDINE NEUROHISTOCHEMISTRY, S.M. Carlton*, Y. Katayama*, G.R. Leichnetz, D.P. Becker and R.L. Hayes. Depts. of Neurosurgery and Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

Following mechanical brain injury, changes in the level of neural activity occur in regions responsible for some of the pathophysiological sequelae of concussion. Studies of regional rates of glucose metabolism employing (^{14}C) deoxyglucose indicate that the ventral tegmental nucleus (VTN) demonstrates a notable increased level of glucose metabolism following low grade mechanical brain injury in the cat. In addition, microinjection of cholinergic agonists into VTN in the cat results in postural atonia, a behavioral state typically seen following concussive injury (Hayes et al, Soc. for Neurosci. Abstr. 8 In press). In the light of these observations, a study of the afferents of VTN was undertaken to identify those sites which could mediate the increased rate of glucose utilization seen in this nucleus after concussive injury.

Four adult cats were stereotaxically implanted with a solid pellet of horseradish peroxidase (HRP) polyacrylamide gel in the VTN and adjacent areas through a previously implanted stainless steel cannula to avoid contamination along the implantation tract. Following a 48 hr. survival period, the animals were perfused with mixed aldehydes (1% paraformaldehyde, 1.25% glutaraldehyde) in 0.1M phosphate buffer, frozen sectioned at 40µ and reacted according to the tetramethylbenzidine protocol of Mesulam ('78).

An abundance of retrogradely labelled cells were observed in the ipsilateral medial and lateral mammillary nuclei (MN), contralateral dorsal tegmental nucleus, and bilaterally in the lateral habenular (Hb) and interpeduncular nuclei. A moderate amount of labelling was seen in the hypothalamus, ipsilateral prerubral fields, ventral periaqueductal gray, interstitial nucleus of Cajal (INC), nucleus of the posterior commissure, trochlear and vestibular nuclei, contralateral deep superior colliculus (dSC), and bilateral medial Hb. A small number of heavily labelled cells were observed in the ipsilateral rostral mesencephalic reticular formation. An occasional labelled cell was present in the ipsilateral oculomotor nucleus. Contamination of the medial longitudinal fasciculus, dorsomedial to the VTN, could account for some of the retrograde labelling in the INC, oculomotor, trochlear and vestibular nuclei, while interruption of tectospinal fibers might explain the scattered labelling in the dSC. The large numbers of heavy labelled neurons in the medial and lateral MN, suggests that they represent the primary source of afferents to VTN.

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47.20 ACTIVATION OF PONTINE CHOLINERGIC SITES IN THE CAT IMPLICATED IN COMA PRODUCED BY MECHANICAL BRAIN INJURY: BEHAVIORAL AND PHYSIOLOGICAL STUDIES. R. L. Hayes, Y. Katayama*, S. M. Carlton* and D. P. Becker. Div. of Neurosurgery, Virginia Commonwealth University, Richmond, VA 23298.

Low levels of mechanical brain injury in the cat produce increased glucose metabolism in the vicinity of the cholinergic pontine ventral tegmental nuclei (Hayes R. L. et al., Neurosci., 11:376, 1981). Other data have suggested that cholinergic activation of this region may produce profound behavioral suppression resembling coma (e.g. Mitler, M. M. and Dement, W. C., Brain Res., 68:335, 1974). Thus we investigated the behavioral, anatomical and pharmacological features of carbachol microinjection in the cat pons. Two guide tubes (26 g) were chronically implanted bilaterally through the cerebellum of 35 cats. After recovery from surgery, awake, unanesthetized cats were loosely restrained in canvas bags. Carbachol was microinjected (0.4-0.6 ug/0.2-0.3 ul, bilaterally) at 4 day intervals through cannulae (33 g) into various pontine loci. Systematic behavioral assessments of motor functions (M) included examinations of EMG, locomotion, flexion and stretch reflexes, resting muscle tone, righting and placing reflexes and pupillary reflexes. Changes in arousal (A) were inferred by studying eye lid, nictitating membrane and pupillary responses to intense stimuli, eye tracking movements, eye blink to visual stimuli and EEG changes. 150 sites were studied. Carbachol microinjection produced maximal M suppression in dorsomedial pontine sites extending from the vicinity of the ventral tegmental (VTN) area caudally to an area slightly ventromedial to locus coeruleus (VM-LC). Within VM-LC, carbachol microinjection produced far greater suppression of M than A. Other sites were associated with equivalent M and A suppression. Microinjection in the gigantocellular tegmental field produced relatively little suppression of M and A. No behavioral effects resulted from microinjection into locus coeruleus. Atropine, either microinjected (0.4-1.2 ug, bilaterally) or systematically administered (0.5-1.0 mg/kg,i.v.), significantly antagonized behavioral effects produced by prior carbachol microinjection. Microinjection of mecamylamine (0.4-1.2 ug, bilaterally), did not antagonized carbachol produced behavioral suppression. These data suggest that activation of muscarinic cholinergic pontine sites in the region of VTN may mediate some components of behavioral suppression seen after mechanical brain injury in the cat. Supported by NIH Grant #NS12587.

48.1 CORTICOSTRIATE PROJECTIONS FROM THE POSTERIOR PARIETAL CORTEX IN RHESUS MONKEYS. E.H. Yeterian and B.A. Leonard*. Department of Psychology, Colby College, Waterville, ME 04901.

Recent autoradiographic investigations have demonstrated that certain regions of the frontal and temporal lobes have more extensive projections to the striatum than had been observed with ablation-degeneration methods. The present study considered the possibility that autoradiography might reveal posterior parietal areas to have more widespread corticostriate projections than previously described, that is, projections to rostral and caudal as well as middle regions of the striatum. In 14 monkeys, tritiated amino acids were injected into various architectonic subdivisions of the posterior parietal cortex as delineated by Pandya and Seltzer (1982). The rostral inferior parietal lobule (IPL), area PE, projects to the lateral head of the caudate nucleus, and to central and ventral portions of the body. In the rostral putamen, projections are strongest ventrolaterally, with some dorsolateral and central extent. More caudally in the putamen, a band of projections extends diagonally from a ventrolateral to a dorsomedial location. The projections of the mid-ventral IPL, areas PG and PGop, were similar to those of area PF, except that none were evident in the lateral putamen. The caudal portion of the dorsal IPL, areas PG and Opt, projects heavily to dorsolateral and ventral regions of the head and body of the caudate nucleus, and to the medial portion of the tail. Projections to the putamen are sparse. The caudalmost IPL, area Opt, projects only to the caudate nucleus, dorsally in the head, dorsally to ventrally in the body, and medially in the caudal bend of the nucleus.

The superior parietal lobule (SPL) has a different distribution of corticostriate projections. The rostral SPL, area PE, projects laterally in the rostral putamen, and dorsally at more caudal levels. Projections to the caudate nucleus are sparse, in lateral regions of the body. The SPL along and within the middle third of the intraparietal sulcus, areas PE and PEa, has similar projections to the putamen, and also a projection to the head of the caudate nucleus laterally. The caudal SPL, area PEc, projects to dorsolateral regions of the head of the caudate nucleus, and along the dorsal and lateral borders of the putamen. Finally, the medial parietal cortex, areas PGm and PEm, has projections to the dorsolateral head and body of the caudate nucleus, and to the adjoining dorsomedial putamen.

These results reveal that posterior parietal projections are distributed broadly throughout the rostral-caudal extent of the striatum. Rostral areas of both the IPL and the SPL project more extensively to the putamen, while caudal areas project more extensively to the caudate nucleus. Inferior parietal projections are, overall, more widespread than those from superior or medial areas.

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48.2 COLLATERAL AXON SYSTEMS WHICH PROJECT UPON THE CAUDATE NUCLEUS, AS STUDIED IN THE CAT WITH THE FLUORESCENT DOUBLE LABELING METHOD. G. James Royce, Dept. of Anatomy, University of Wisconsin, Madison, WI 53706

The retrograde fluorescence technique was used to label single neurons within both subcortical and cortical regions which, in addition to projecting to the caudate nucleus, also project to other structures. After experimentation with many other pairs of fluorescent tracers, Evans Blue (EB) and Fast Blue (FB) were chosen as the best combination for studying the systems involved. The tissue sections were examined with a Nikon Fluophot microscope which is electrically coupled to an X-Y plotter.

Following injections of EB into the caudate nucleus and FB into the rostral cortex (gyrus proreus) doubly labeled neurons are present within several thalamic nuclei which include the ventral anterior, ventral lateral and mediodorsal nuclei. Doubly labeled cells are also present in most members of the intralaminar group, including the central medial, paracentral, central lateral and parafascicular nuclei. Although the centromedian nucleus contains large numbers of singly labeled EB cells which project to the caudate nucleus, and a few FB cells which project to the cortex, doubly labeled neurons are absent from this posterior intralaminar nucleus, in this series of experiments.

In a separate series of experiments, EB was injected into the caudate nucleus and FB was injected into the centromedian and parafascicular nuclear complex. Following such injections, doubly labeled neurons are present within the cerebral cortex, where they are confined to layers V and VI. These doubly labeled cells are medium-sized pyramidal neurons, which are found within areas 4 and 6, in the anterior limbic area, in the cingulate and anterior sylvian gyri, and within the buried cortex of the presylvian sulcus.

This study was supported by NIH grant NS13453.

48.3 ISOLATION OF THE MONOSYNAPTIC THALAMO-STRIATAL EPSP IN SPINY PROJECTION NEURONS OF RAT CAUDATE-PUTAMEN. K. D. Phelan*, C. J. Wilson, H. T. Chang and S. T. Kitai. (SPON: Sharleen Sakai). Department of Anatomy, Michigan State University, East Lansing, MI 48824.

Stimulation of thalamic intralaminar nuclei or structures along the intrathalamic trajectory of thalamo-striatal axons evoked complex EPSPs and subsequent hyperpolarization in rat neostriatal spiny neurons identified by intracellular injection of horseradish peroxidase. The initial EPSP portion of the response, which could be as much as 75 ms in duration, was seen to consist of several components. In intact urethane and ketamine anaesthetized rats, latency variations suggestive of a polysynaptic origin could often be demonstrated even for early (2-10 ms) EPSP components. Individual components of the excitatory response could not be clearly distinguished in most neurons however, and the earliest excitatory component usually appeared to be monosynaptic.

After large acute aspiration lesions of ipsilateral cerebral cortex, early polysynaptic EPSP components of thalamic-evoked EPSPs were absent or greatly attenuated. This suggested that most or all of the short latency polysynaptic EPSP components arose via a thalamo-cortico-striatal route. A short latency (1.6-4.0 msec) monosynaptic EPSP and a second excitatory component with a more variable latency (8-28 ms) remained intact after acute decortication. These were not dependent upon intact cortico-thalamic or cortico-striatal axons, since they were both still present in experiments performed as long as 4 days following ipsilateral hemidecortication. The longer latency excitatory response was shown to be polysynaptic by its latency variation with changes in stimulus intensity and frequency. This component of the response was abolished after acute thalamic hemitransections separating thalamo-striatal neurons from their axons. In these experiments, stimulation of thalamo-striatal axons rostral to the transection continued to evoke monosynaptic EPSPs in striatal spiny neurons. These EPSPs ranged from 1.8-3.0 ms in latency, had peak amplitudes at latencies of from 7-12 ms, were 20-37 ms in duration and attained peak amplitudes up to 11 mV. This EPSP is interpreted as representing the sole monosynaptic effect of thalamo-striatal afferents to spiny projection neurons. [Supported by NIH Grants NS 17294 (to C.J.W.) and NS 14866 (to S.T.K.)].

48.4 A GOLGI-ELECTRON MICROSCOPIC STUDY OF TWO TYPES OF LARGE NEURONS IN THE MONKEY NEOSTRIATUM. J. Carey* and M. DiFiglia. Dept. of Neurology, Mass. General Hospital, Boston, MA 02114

Golgi studies in the monkey neostriatum (DiFiglia et al, Brain Res., 114, 1976) have shown that at least two types of large neurons are present. Aspiny type 11 neurons have been identified by their large (30-40 μm) round or ovoid somata and numerous smooth surfaced varicose dendrites which curve around the cell body and extend up to 250 μm away. The spiny type 11 neuron has an elongated soma (30-40 μm in length) and few thick sparsely branching spiny dendrites which extend long distances (up to 600 μm).

In the present study neurons belonging to the aspiny type 11 (N=4) and spiny type 11 (N=1) categories were selected from Golgi impregnated monkey (M. fascicularis; N=2) caudate tissue which had been gold-toned according to the protocol of Fairen et al (J. Neurocytol., 5, 1977) and embedded in Epon for ultrastructural study. Results showed that aspiny type 11 neurons had a round, eccentrically positioned nucleus, which at most levels exhibited numerous shallow indentations along its inner face. The cytoplasm at all levels was rich in organelles and contained many single strands and some large stacks of rough endoplasmic reticulum (RER). Primary dendrites had abundant microtubules arranged in parallel arrays and the varicosities present in distal branches contained numerous mitochondria. Synaptic contacts were infrequent on the cell body and proximal dendrites and more numerous on distal branches where they appeared mostly on varicosities. Up to four boutons could be seen contacting the same varicosity. The most numerous were about 1 μm in size, contained pleomorphic vesicles and formed symmetric synapses. Boutons with round or pleomorphic vesicles formed asymmetric synapses and also contacted nearby dendritic spines. The spiny type 11 neuron had an elongated, centrally located nucleus. At some levels the nucleus showed no indentations and in other locations exhibited a few shallow and deep enfoldings. The cytoplasm was rich in organelles and contained small stacks of RER and a well developed Golgi apparatus which rimmed various portions of the nucleus. Synapses were observed relatively frequently on the cell body and dendritic shafts and were formed by axon terminals of different types which made either symmetric or asymmetric contacts. Dendritic spines always appeared postsynaptic and were contacted by boutons with round or pleomorphic vesicles which made asymmetric synapses. The axon initial segment also received many synaptic inputs. The present electron microscopic results support Golgi studies that distinguish two classes of large neostriatal neurons in the monkey and demonstrate differences in the pattern of distribution of synaptic inputs to these cells. (Supported by NIH grant NS 16367).

48.5 NEURONAL ACTIVITY IN THE VENTRAL STRIATUM OF THE BEHAVING MONKEY. E.T.Rolls, J.Ashton*, G.Williams*, S.J.Thorpe*, G.J.Mogenson, F.Colpaert*, and A.G.Phillips. University of Oxford, Department of Experimental Psychology, Oxford, England.

The ventral striatum of the primate receiyes projections from structures such as the amygdala, hippocampus and inferior temporal visual cortex (ITC). To analyse the functions of the ventral striatum, the activity of more than 400 single neurons was recorded in a region which included the nucleus accumbens and olfactory tubercle in 4 macaque monkeys in test situations in which lesions of the amygdala, hippocampus and ITC produce deficits. The following types of neuronal response have so far been identified. First, there are neurons which respond to novel visual stimuli. Different neurons in this category show pattern-specific habituation over 1-10 trials, and show retention of this habituation over 1-14 intervening trials. A small number of neurons respond to familiar rather than to novel visual stimuli. Second, other neurons respond to visual stimuli of emotional significance. Many of these neurons respond to faces and some other aversive stimuli, some respond to certain rewarding stimuli, for example to some foods, some respond to both rewarding and aversive visual stimuli, and other neurons respond with opposite changes of firing rate to aversive and rewarding visual stimuli. These neurons do not respond simply in relation to arousal, produced by inputs from different modalities. Third, other neurons respond differentially in a visual discrimination task, either to the stimulus associated with punishment or to that associated with reward. Fourth, other neurons respond in relation to somatosensory stimuli or movement. Fifth, other neurons respond, as in the head of the caudate nucleus (Rolls et al, 1979), to cues such as a 0.5 sec tone which the monkey uses to prepare for the performance of the visual discrimination task. Sixth, other neurons responded in relation to arousal, however this was produced. A number of neurons responded in more than one category, responding maximally for example to relatively novel aversive stimuli. These findings are consistent with the hypothesis that the ventral striatum is involved in certain behavioral responses to novel and emotion-provoking visual stimuli, which influence it through its inputs from limbic structures.

Rolls,E.T., Thorpe,S.J., Maddison,S., Roper-Hall,A., Puerto,A. and Perrett,D. (1979) Activity of neurones in the neostriatum and related structures in the alert animal. Pp 163-182 in The Neostriatum, eds I.Divac and R.G.E.Oberg. Oxford: Pergamon Press.

48.6 DENDRO-DENDRITIC SYNAPSES IN SUBSTANTIA NIGRA: QUANTITATIVE DESCRIPTIONS BASED ON SERIAL ELECTRON MICROGRAPHS. J.C. Linder*, S.J. Young* and P.M. Groves (SPON: D.S. Segal). Dept. of Psychiatry, Univ. Calif. San Diego, La Jolla, CA 92093.

Recent evidence has established that dopaminergic neurons of the substantia nigra release dopamine from their dendrites, presumably regulating dopaminergic neuronal activity and the release of neurotransmitters from afferent and efferent pathways. An important unresolved question, however, has been the mode of release of dopamine from these dendrites. This study has analyzed dendro-dendritic synapses in serial sections of substantia nigra from rats whose catecholaminergic synapses had been labeled with intraventricular 5-hydroxydopamine.

Distinct dendro-dendritic synapses, all labeled by 5-hydroxydopamine, were found only in pars compacta, but were rare even in that region of substantia nigra. Quantitative descriptions were based on 29 dendro-dendritic synapses made by 15 serially-sectioned (14-52 sections/series) presynaptic dendrites. These synapses were all very small, having a mean junctional length of 322±23nm and appearing in only 2-23 adjacent 80nm sections. The synaptic clefts averaged 15±0.8nm in width and were bordered by delicate symmetrical membrane specializations. A small number (mean=32±5) of labeled pleomorphic synaptic vesicles (30.7±0.7nm in diameter) were clustered against the junctional membrane. No reciprocal or serial synapses were found.

The morphology of several dendro-dendritic synapses has been visualized by computer-assisted three-dimensional reconstruction and compared to reconstructions of axo-dendritic synapses in the substantia nigra. Presynaptic dendrites had diameters of 0.23-1.9µm and did not change calibre at the site of the synapse, in contrast to axons which had terminal enlargements. Presynaptic dendrites did not receive other synapses near the dendro-dendritic junctions. They frequently made several dendro-dendritic synapses onto different postsynaptic dendrites within a short distance, but then remained closely apposed to the adjacent dendrite for several micrometers with no further synaptic junctions. The postsynaptic elements of these dendro-dendritic synapses closely resembled the presynaptic dendrites. They were of similar diameter and also received very few axo-dendritic synapses. In 4 instances, it was possible to show that the postsynaptic dendrites were also labeled by 5-hydroxydopamine, indicating that some dendro-dendritic synapses exist between dopaminergic neurons. The results of this ultrastructural study suggest that these dendro-dendritic synapses contribute to the dendritic release of dopamine in the substantia nigra.

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48.7 EFFERENT CONNECTIONS OF THE VENTRAL PALLIDUM IN THE RAT. S.N. Haber, H.J. Groenewegen* and W.J.H. Nauta, Dept. of Psychology and Brain Science, Mass. Inst. of Technology, Cambridge, MA 02139 and Mailman Research Center, McLean Hospital, Belmont, MA 02178.

Recent findings suggest a subdivision of the striatum into a. a ventromedial region (including the nucleus accumbens and olfactory tubercle) receiving partially overlapping projections from hippocampus, amygdala, ventral tegmental area, mesencephalic dorsal raphe nucleus, and prefrontal cortex (Kelley et al., Neuroscience 7, 615-630), and b. a dorsolateral region that receives a massive projection from the somatosensory cortex but is either avoided or only sparsely innervated by the afferents from the limbic-associated structures enumerated above. The proposed subdivision thus contrasts a limbic-innervated with a somatic cortex-innervated district of the striatum. Hitherto unpublished observations in this laboratory indicate that the striatopallidal projection from the limbic-afferented region of the striatum involves almost exclusively the pallidal compartment called the ventral pallidum (vp), while that from the non-limbic region exclusively involves the main body of the pallidum or dorsal pallidum (dp). We here report an autoradiographic study of the projections from the subcommissural portion of the vp as demonstrated by anterograde transport of tritiated amino acids, with control experiments involving surrounding structures such as the bed nucleus of the stria terminalis, lateral preoptic area, nucleus of the diagonal band and adjoining striatum. Preliminary findings indicate that the subcommissural vp projects not only to the most medial part of the subthalamic nucleus and to the substantia nigra, but also to the amygdaloid complex (mainly nucleus basalis lateralis), the mediodorsal nucleus of the thalamus, and the lateral habenular nucleus. Less unambiguous evidence suggests possible further vp projections to the peribrachial region, lateral hypothalamus, reticular and ventral anterior nuclei of the thalamus, nucleus accumbens and prefrontal cortex. Considered together, these findings indicate a conduction line leading from the limbic-afferented striatal subdivision via the ventral pallidum both into somatic motor circuitry (subthalamic nucleus), as well as back into the circuitry of the limbic system.

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48.8 FLUORESCENT RETROGRADE STUDY OF THE PALLIDOINTRALAMINAR AND PALLIDONIGRAL PATHWAYS IN PRIMATE. L. De Bellefeuille* and A. Parent. Lab. of Neurobiology, Fac. Med., Laval Univ., Québec, Canada, G1K 7P4.

In regard to its thalamic, habenular and midbrain efferents, the primate internal pallidal segment (GPi) was shown to be organized according to a complex pattern consisting of 3 concentric zones: (1) a large central 'motor' zone where most neurons send axonal branches to both thalamus and midbrain, (2) a small peripheral 'limbic' zone which encroaches largely upon the lateral hypothalamus and whose neurons project only to habenula, and (3) a peripallidal 'reticular' zone composed of large acetylcholinesterase-containing cells related to nucleus basalis and projecting diffusely to neocortex and to a lesser extent to brain stem (Parent and De Bellefeuille, Brain Res. 1982).

In an attempt to further our knowledge of the organization of GPi, Evans blue (EB) was injected into the VA/VL thalamic nuclei and DAPI-primuline mixture (DP) in centre-médian/parafascicular intralaminar complex (CM/Pf) in 4 squirrel monkeys (Saimiri sciureus). In a second series of experiments 7 squirrel monkeys received EB injections in VA/VL and DP injections in the substantia nigra (SN).

In the first series of experiments most of the CM/Pf-projecting cells were found to be clustered in the dorsolateral and central portion of ipsilateral GPi, whereas the VA/VL-projecting cells were much more abundant and more diffusely distributed in the same structure. About 65% of all the fluorescent pallidal cells were double-labelled, 25% were labelled only with EB (VA/VL-projecting cells), and 10% only with DP (CM/Pf-projecting cells). A few scattered CM/Pf-projecting cells were also found in contralateral GPi. In addition, a significant number of retrogradely-labelled cells was found in the ipsilateral superior colliculus (stratum griseum medium), in the nucleus tegmenti pedunculopontinus pars compacta and diffusa, bilaterally, and in ipislateral nucleus reticularis thalami and zona incerta, after CM/Pf injections. In the second series of experiments, VA/VL injections resulted in a profuse retrograde labelling of GPi cells, as in the monkeys of the first experimental group. In contrast, the GPi appears devoid of DP-labelled cells even after rather massive SN injections. However, a significant number of SN-projecting cells was found in the dorsolateral portion of the external pallidal segment (GPe).

In conclusion, our findings suggest that the pallidointralaminar and pallidothalamic pathways arise mostly from the same neurons in the lateral part of GPi, whereas the pallidonigral projection seems to originate exclusively from the GPe.

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48.9 PICROTOXIN IN THE STRIATUM INCREASES FIRING RATE IN THE VENTROMEDIAL NUCLEUS OF THE THALAMUS (VMT).
P. Patino* and M. Garcia-Munoz. (SPON: J. Garcia-Ramos) Dept. of Neuroscience, Cellular Physiology Research Center, National University of Mexico, 04510 Mexico,D.F.

Recent studies propose that activation of a GABAergic system which projects from substantia nigra may be involved as an output system for nigro-striatal responses. Studies on turning behaviour induced by substantia nigra pars compacta lesion, indicate that VMT is among the structures involved in this behavioural expression after dopamine receptor activation.

We have investigated the extracellular activity of cells in VMT of control and 6-OHDA lesioned rats. The mean firing rate of control cells is 0.9+0.01 spikes/sec. After amphetamine 0.1 mg/kg i.v. it increases to 8.1+0.04 spikes/sec. However this effect is not observed after intrastriatal injection of amphetamine (20 ng/0.5 µl) or apomorphine (20-200 ng/1 µl). Nevertheless picrotoxin (200 ng/1 µl) or glutamate (200 ng/1 µl) locally injected into the striatum does increase VMT firing rate.

This study provides further evidence for a possible relationship between the striatum, and VMT, which could be mediated through the substantia nigra or the globus pallidus.

48.10 BEHAVIOURAL CHANGES INDUCED BY ALTERING GABA TRANSMISSION IN THE THALAMUS.
M. Garcia-Munoz, A. Zainos* and L. Chavez*. Dept. of Neuroscience, Cellular Physiology Research Center, National University of Mexico, 04510 Mexico, D.F.

It has been suggested that the striato-nigral pathway and the substantia nigra reticulata efferents are important for the influence of striatal dopamine receptors on behaviour. GABA seems to be the major neurotransmitter of this output system.

We have investigated the effect of manipulating GABA transmission in the ventromedial (VM) and parafascicular (PF) nucleus of the thalamus which receive a gabaergic input from the substantia nigra reticulata. Muscimol (250 ng/0.5 µl) locally injected in VM or PF induces catalepsy. Picrotoxin (250 ng/0.5 µl) in VM or PF impairs the animal's orienting response towards an electrically stimulated site in the skin (1-5mA / 60 sec). Picrotoxin in VM alone induces $\bar{X}$=74±30 contralateral turns/30 min and stereotyped upward sniffing. If picrotoxin is injected in PF, the turning is ipsilateral and faster $\bar{X}$=74±30 turns/30 min. The blockade of GABA receptors in the parafascicular nucleus also produces a new motor expression consisting of an ipsilateral barrel rolling $\bar{X}$=318±40 turns/30 min.

This study provides evidence for two possible components of the motor output, one involved in the locomotion in circles and the other in the maintenance of balance and posture.

48.11 SOURCES OF INFORMATION CONVERGING WITH BASAL GANGLIA OR CEREBELLAR INPUT TO THE THALAMUS.
M.E. Anderson and J.L. DeVito. Depts of Rehab. Med., Physiol. and Biophys., and Biol. Struct. and Regional Primate Res. Cent., Univ. of Wash., Seattle, WA 98195.

There is considerable evidence to suggest that output from the basal ganglia (BG) and cerebellum (CB) remains separated at the thalamus. To identify potential sources of information converging with BG or CB output at the thalamus, we have made small injections of horseradish peroxidase into different areas of the ventroanterior and ventrolateral thalamus in cats and quantitatively assessed the relative retrograde labeling of neurons in the basal ganglia and deep cerebellar nuclei. We then compared the locations of cortical and subcortical neurons labeled when HRP was injected into relatively "cerebellar" or "basal ganglia" thalamus.

When HRP was injected into the most rostral portions of VA, the ratio of BG:CB cells labeled was high, and in the cerebral cortex, HRP labeled cells were restricted to ventral portions of gyrus proreus, the prefrontal regions. Outside of ENTO and SN, no labeled neurons were found in the brainstem, including the superior colliculus (SC) or pedunculopontine region (PPN). When HRP was injected more caudally into VL (A 11.0), the ratio of BG:CB neurons labeled depended on the ventromedial to dorsolateral position of the injection. With ventromedial injections, which labeled more BG than CB, neurons labeled in the cortex were located in the ventral prefrontal areas and in area 6, but there was little label in area 4. Labeled neurons also were found in SC and PPN. As the injections progressed dorsolaterally, more CB cells were labeled and, in the cortex, fewer cells were labeled in the prefrontal regions and more in area 4. In the brainstem, labeled neurons were in the pretectum, with only a few in PPN and SC.

When injections were located even more caudally in VL, sometimes extending into prominent portions of the ventrobasal complex the number of BG cells labeled dropped markedly, and, in the cortex, most labeled cells were in area 4. In the brainstem, few labeled cells were present in SC, a few were present in the red nucleus, but none were seen in PPN.

These data show that sources of input converging with basal ganglia information at the thalamus are cortical areas 6 and the gyrus proreus, the superior colliculus or pretectal areas, and the PPN region. Thalamic areas receiving input from cerebellar nuclei receive information from cortical area 4 and perhaps 6 and the red nucleus, but probably not from the superior colliculus or PPN. There also may be a further suborganization of corticothalamic relationships or convergence of BG and CB input to restricted areas of the thalamus.

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48.12 ELECTRON MICROSCOPIC AUTORADIOGRAPHY OF PALLIDOTHALAMIC TERMINALS IN THE CAT.
K. Kultas-Ilinsky, I. A. Ilinsky and K. R. Smith. Depts. of Anatomy and Surgery, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

The entopeduncular nucleus (EPN), - feline homologue of the primate medial segment of the globus pallidus, is the main source of pallidothalamic projections. The precise thalamic area receiving projections from the EPN was reported in a previous light microscopic autoradiography study (Ilinsky et al., 1982). In the present investigation, we used electron microscopic autoradiography for the ultrastructural identification of pallidal terminals in the thalamus to determine their synaptic sites. The animals received 0.1-0.3 uL injections of 2,3,4,5[^{3}H]-leucine (70 uCi/ul) in the EPN and after 4-5 days survival they were perfused for electron microscopy. Tissue samples were removed from the ventral medial and ventral anterior thalamic nuclei and processed for electron microscopic autoradiography as described earlier (Kultas-Ilinsky et al., 1980).

The majority of labelled pallidal boutons were of medium size (2.0-5.0 um in length along the postsynaptic membrane and 1.0-1.5 um in height). In a number of instances favorable section planes revealed the en passant nature of pallidal boutons. Profiles of labelled terminals contained large numbers of uniformly distributed small clear vesicles of variable shape, as well as variable amounts (1-10) of large dense core vesicles. The average mean diameter of clear synaptic vesicles was 36 nm and the average ellipticity index (Dl/Ds) was 1.6. Pallidal boutons in all instances formed symmetrical type synaptic contacts. Multiple synaptic junctions between an individual bouton and postsynaptic structure were very frequent. Synapses were invariably on dendritic shafts and never on spines. Quantitative analysis of the distribution of synaptic sites showed that 40% of pallidal synapses were on somata and primary dendritic branches of thalamocortical projection neurons, 36% on secondary dendrites, 13% on tertiary dendrites. Eight percent of labelled boutons formed synapses on vesicle-containing dendrites of local circuit neurons. An interesting feature of the distribution of pallidal terminals was their tendency to be located at bifurcation sites of the dendrites of thalamocortical projection neurons. For example, 37% of all the terminals on primary dendrites were located at the branching points. Our data indicate that pallidal activity reaches both types of cells in the thalamus: thalamocortical projection neurons and local circuit neurons, though in different proportions. On the thalamocortical projection neurons pallidal terminals occupy a strategically important positions which would enable them to control the activity arriving at the thalamus via other afferent systems. (Supported by NINCDS grant #IROI NS17388 -01 and a grant from the American Parkinson Disease Association.)

48.13 THALAMO-SUBTHALAMIC PROJECTION: AN AUTORADIOGRAPHIC STUDY. T. Sugimoto*, N. Mizuno and T. Hattori. Dept. Anat., Fac. Med., Univ. Toronto, Med. Sci. Bldg, Toronto, Ont. M5S 1A8 Canada and Dept. Anat., Fac. Med., Kyoto Univ., Kyoto 606 Japan.

In the course of studies on thalamic projections in the rat and cat using anterograde fiber tracing methods, a substantial, yet previously unreported projection from the centre median-parafascicular nuclei (CM-Pf) to the subthalamic nucleus (STN) was observed. Small injections of 3H-leucine were made stereotaxically into the CM-Pf complex in rats. In cats, a mixture of 3H-leucine and 3H-proline was injected into the CM-Pf. Control injections were made intentionally at several neighboring thalamic nuclei including the ventral, medial or intralaminar nucleus. Tissue from all animals was processed using standard autoradiographic procedures. In all animals which were injected with isotope into the CM-Pf complex, the ipsilateral STN was heavily labeled over its rostral one-fourth. Thus, label was most concentrated within the rostral pole of the STN, became more sparse caudally, and disappeared over the caudal half of the STN. The rostral pole of the rat STN was quite diffusely labeled. In the cat, however, radiolabel was distributed mainly in the ventral or ventromedial portions of the STN, especially the portion intermingled with labeled fibers of passage bordering the ventral aspect of the STN. These clearly identified labeled fibers of passage consisted of several groups of fiber bundles which appeared to run in the cerebral peduncle and approached the ventral aspect of the STN. At the middle levels of the STN heavily labeled fibers of passage were seen along the ventral and dorsal boundaries of the STN, encircling the convex-shaped, sparsely labeled nucleus. In control experiments, in which isotope was injected into the ventral, medial or intralaminar thalamic nuclei, no prominent accumulation of silver grains occurred within the STN. Thus, the CM-Pf fibers projecting to the STN were not distributed to the middle nor caudal levels of the STN. The restricted rostral distribution pattern of label in the STN may be the reason why previous HRP studies (Rinvik et al., '79, in cat and monkey; Carpenter et al., '81, in monkey) failed to reveal any thalamic afferents to the STN. The CM-Pf complex has widespread projections to the striatum as well as the projection described here to a restricted region of the STN. The CM-Pf is thus in a position to exercise control over divergent portions of the basal ganglia. (Supported by the grants from the Medical Research Council of Canada).

48.14 A GOLGI STUDY OF THE NEURONS IN THE CENTROMEDIAN-PARAFASCICULAR COMPLEX IN THE LESSER BUSHBABY (GALAGO). J. C. Pearson*, C. H. Phelps, and J. R. Norris* (SPON: G. Crampton). Dept. of Anat., Wright State Univ. Sch. of Med., Dayton, OH 45435

In the present study the morphology of neurons in the centromedian-parafascicular (CM-PF) complex of three adult lesser bushbabies (Galago senegalensis) was examined using the zinc chromate modification of the Golgi method. All forebrains were cut in a transverse plane oriented approximately perpendicular to the longitudinal axis of the brainstem. Nuclear configuration of the CM-PF complex was confirmed by comparison of Golgi stained sections with transverse forebrain sections processed according to cell body and fiber stains. Two basic cell types were identified in the centromedian nucleus, and designated as principal neurons or interneurons (Golgi type II neurons). Somata of principal neurons were round to oval in shape, and from 15-22μm in diameter. Each cell gave rise to 2-5 primary dendrites which were of unequal thickness, infrequently branched, and appeared to diverge from the cell body in a radiate type of branch pattern similar to that previously described for reticular formation neurons. Some dendritic processes extended long distances from the somata (more than 200μm) and were oriented in a ventromedial to dorsolateral plane parallel to the direction of the fibers in the interal medullary lamina. The dendrites contained a moderate amount of spines which consisted of small bulbous spheres (less than 1μm diam.) supported by short (approx. 2μm) slender stalks. The spines appeared to be arranged in serial order with no apparent clustering. Axons appeared as short partially impregnated structures (approx. 20-40μm long) which often arose from the proximal portions of primary dendrites. Golgi type II neurons in the centromedian had rounded cell bodies approximately 11-14μm in diameter. Each cell gave rise to 2-4 dendrites which were relatively long (approx. 150-200μm) and infrequently branched. The dendrites supported appendages which were often long (30μm) and intricately branched into many independent lobules or swellings which resembled multilobulated appendages described in previous Golgi studies of many regions of the CNS. Some dendritic branches did not support bulbous appendages, but rather, contained evenly spaced swellings which gave the presumed dendritic process an axon-like appearance. In the parafascicular nucleus several principal neurons with somatic spines were observed. Dendrites of these cells supported spines which were similar in morphology to those observed on dendrites of principal cells in the centromedian. Axons appeared partially impregnated and frequently arose from primary dendritic processes. (Supported by RI-RD funds awarded by the WSU Research Council.)

48.15 A GOLGI STUDY OF THE NEURONS IN THE SUBTHALAMIC NUCLEUS IN A PROSIMIAN (GALAGO). C. H. Phelps, J. C. Pearson*, and J. R. Norris*. Dept. of Anat., Wright State Univ. Sch. of Med., Dayton, OH 45435

The morphology of neurons in the subthalamic nucleus of three adult prosimian primates (Galago senegalensis) was studied using the zinc chromate modification of the Golgi method. Impregnated sections were cut in a transverse plane oriented approximately perpendicular to the long axis of the brainstem. The location of impregnated neurons in the subthalamic nucleus was confirmed by comparison of Golgi stained sections with cell-body and fiber stained brain series. The present results indicate two kinds of principal neurons, and small cells considered to be interneurons in Galago subthalamic nucleus. Because of several points of similarity with principal neurons previously described in the subthalamic nucleus of Macaca mulatta (Rafols and Fox, JCN,168: 75-112, 1976), the principal neurons in Galago were accordingly designated as "radiating neurons" or "elongated fusiform neurons." Radiating neurons had ovoid somata (15-25μm diam.) which supported many fine somatic spines 2-4μm in length. Each somata gave rise to 4-7 primary dendrites which branched infrequently and supported a variable number of spines. Most spines consisted of small bulbous spheres supported by short stalks and were concentrated along the proximal dendritic regions. The dendrites also gave rise to many beaded axon-like processes which extended for distances of up to 80μm from their dendritic origin. The dendrites extended for long distances (approx. 400μm) from the cell bodies and demonstrated a radiate type branch pattern. Some dendritic processes extended into the overlying lenticular fasciculus, and many were conspicuous in their considerable extension (greater than 200μm) into the ventrally located crus cerebri. Elongated fusiform neurons were less frequently found than radiating neurons, and were often located along the dorsal aspect of the nucleus or immediately adjacent to the crus cerebri. Their somata were fusiform in shape, free of spines and gave rise to from two to five primary dendrites. The dendrites supported fewer spines than did those of the radiating neurons, and they often extend greater distances from the somata. Axons of both principal cell types were impregnated only in what appeared to be the initial segments. Presumed interneurons had small, round cell bodies (10-15μm diam.) and few infrequently branched and extrememly sinuous dendrites. These dendrites gave rise to many elaborately branched appendages and supported many beaded, axon-like processes. (Supported by research development funds awarded by the WSU Research Council.)

48.16 A GOLGI STUDY OF RAT SUBTHALAMIC NUCLEUS. Salman Afsharpour*, H. Imai and S. T. Kitai. Department of Anatomy, Michigan State University, East Lansing, MI 48824.

The morphology of the subthalamic nucleus (STH) of Sprague-Dawley rats was studied by the Golgi techniques (Ramon-Moliner's modification, 1957; Valverdé, 1970; Gobel, 1978). Sections were cut either in sagittal or coronal planes. Golgi impregnated neurons were analyzed under a light microscope and were drawn with the aid of a drawing tube. Some representative neurons were also photographed. The cross-sectional areas of the soma (SA) were measured with the aid of a graphic analyzer attached to a PDP-11 computer.

The soma shape varied between elongated fusiform, polygonal and round. The SA varied between 140-440 μm^2. Some of the cells had a few somatic spines. Two to six proximal dendrites gave rise to tapering dendrites which extended up to 500 μm in length. These dendrites were sparsely covered with spines.

Neurons located in the central portion of STH had oval dendritic fields whose long axis was parallel to the long axis of the nucleus in either frontal or sagittal planes. Some of these neurons had one or two dendrites which crossed the borders of STH into the zona incerta, the lateral part of the hypothalamus or the cerebral peduncle (CP). Most neurons located at the borders of STH in general had their dendritic fields extended parallel to the border and were confined within the nucleus except those cells adjacent to CP which had some of their dendrites extending into CP.

Two types of afferent fibers were observed to terminate within STH. One type of fibers were axon-collaterals arising from CP, and the other were fibers that crossed the internal capsule and entered STH rostrally. There were many fibers passing through STH without giving rise to any axon-collaterals.

These results suggest that contrary to the other species (i.e. man, monkey, cat) the rat STH is an open nucleus and that STH may consist of varieties of only Golgi type I neurons. (Supported by USPHS Grant 14866 to S.T.K.)

48.17 SUBTHALAMIC NUCLEUS OF THE RAT: AN ELECTRON MICROSCOPIC STUDY. H. T. Chang, H. Kita and S. T. Kitai. Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

As a part of parallel physiological and anatomical studies of the subthalamic nucleus (STH) in our laboratory, we report here the study of the normal ultrastructural morphology of the rat STH. Adult male albino and hooded rats under deep anaesthesia were perfused with saline and followed by 1% formaldehyde and 2% glutaraldehyde in phosphate or bicarbonate buffer. Brains were cut with Vibratome and STH was prepared for EM analysis.

The overall appearance of STH was dominated by large numbers of myelinated fibers (diameters, D, ranged from 0.3 to 1.5 μm) in the neuropil within which were dispersed tightly packed cell bodies. The neuronal somata were often closely apposed to each other without any intervening glial membranes. Membrane appositions were also found between somata and dendrites as well as dendrites and dendrites. Although puncta adherence junctional complexes were observed, no gap junctions were found on any of these membrane appositions.

The STH cell bodies (D between 10 and 25 μm) were characterized by abundant organelles. Some cells had cilia. The nuclei had deeply invaginated membrane and pale nucleoplasm with little heterochromatin. Filamentous nuclear rods were often observed. In the neuropil, the dendrites were mostly thin (D between 0.5 to 1 μm) with an occasional stubby spine. Both Gray's type I and type II axon terminals were found in STH: The type I terminals formed asymmetrical synapses mainly with thin dendrites and spines. These terminals (D ranged from 0.2 to 1 μm) contained medium-sized round vesicles (D up to 45 nm) and often formed synapses with two postsynaptic targets. The type II terminals formed symmetrical synapses mainly with somata and large dendrites, including the spines on both of these structures. These terminals were often large (D up to 5 μm) and contained large pleomorphic vesicles (largest D up to 50 nm). Frequently these terminals formed adherence junctions with the postsynaptic membrane in addition to the synaptic junctional complexes. Some type II terminals arose directly from myelinated axons, indicating a fast conduction of signals from this afferent source. A third group of terminals included morphological characteristics intermediate between the above two types of terminals, and may represent tangentially sectioned terminals of both types as well as terminals from either intrinsic collaterals of STH cells or other afferent sources.

It is likely that the cortical excitatory inputs and the pallidal inhibitory inputs to STH observed in our physiological studies were mediated by the type I and II terminals respectively.

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48.18 LIGHT MICROSCOPIC OBSERVATION OF RAT SUBTHALAMIC INTRACELLULARLY-STAINED NEURONS. H. Kita, H. T. Chang and S. T. Kitai. Department of Anatomy, Michigan State University, East Lansing, MI 48824

Neurons in the subthalamic nucleus (STH) were intracellularly recorded using HRP-filled microelectrode. After the completion of electrophysiological analysis (which will be presented in a separate paper), some neurons were intracellularly-labeled with HRP. At the end of an experiment, animals were fixed with a mixture of 1% formaldehyde and 2% glutaraldehyde. The brains were cut into 50 μm serial sagittal sections and reacted with the diaminobenzidine.

Light microscopic analysis of HRP-stained STH neurons revealed that: 1) The axons of all analyzed neurons were traced beyond the borders of STH, indicating that they were projection neurons. 2) The somata were polygonal or oval in shape and usually three or four primary dendrites arose from the soma. Occasionally a few spines were seen on the soma. Dendritic trunks tapered slightly and divided into long thin sparsely spined branches. 3) The neurons could be divided into two groups based on their axonal morphology; one with intranuclear axon collaterals and the other without. Differences were also observed in the ratio of their dendritic branching pattern between these two groups: neurons with intranuclear collaterals had higher dendritic tip/stem ratio than the neurons without intranuclear collaterals. 4) Reconstruction of the dendritic field was made in three different planes. In either sagittal or frontal planes, the dendritic field was usually oval in shape and the long axis was parallel to the main axis of STH. In the horizontal plane, the dendritic field of all the neurons were polygonal in shape. 5) All the parent axons originated from the soma ran dorsolaterally and bifurcated at least once. After bifurcation, one axon ran rostrolaterally within the cerebral peduncle and terminated in the globus pallidus. The other collateral coursed caudally or mediocaudally and terminated in the substantia nigra. 6) Some of the globus pallidus-bound axons emitted fine axon collaterals within the entopeduncular nucleus. 7) The terminal arborizations observed in all places (i.e. globus pallidus, entopeduncular nucleus, STH and substantia nigra) were formed with varicose collateral branches which also gave rise to short filaments with beaded endings.

These observations indicated that rat STH comprises of at least two groups of projection neurons with distinguishable axonal and dendritic morphology. The information from single STH neurons could be sent to the globus pallidus, the substantia nigra, and in some neurons, to the entopeduncular nucleus as well as STH itself.

(This study was supported by NIH Grant 14866 to S.T.K. and NRSA 1F32NS06951-01 to H.T.C.)

48.19 IMMUNOCYTOCHEMICAL STUDY TO IDENTIFY NEOSTRIATAL PROJECTION NEURONS AND THEIR PUTATIVE NEUROTRANSMITTERS, SUBSTANCE P AND METHIONINE ENKEPHALIN. Ronald H. Bradley and S. T. Kitai. Department of Anatomy, College of Osteopathic Medicine, Michigan State University, East Lansing, MI 48824.

Morphology of the neostriatal projection neurons has been previously identified by Golgi gold toning, HRP retrograde labeling, or intracellular labeling with HRP. In this study we intend to identify the neostriatal projection neurons and their putative neurotransmitters by immunocytochemical methods in the rat brain.

In order to retrogradely label the neostriatal projection neurons, n-acetyl-WGA was stereotaxically injected into substantia nigra or globus pallidus. After 48 hours post-injection period, animals were again injected stereotaxically with colchicine (10 μg/ml) in the lateral ventricle contralateral to the substantia nigra or globus pallidus previously injected with WGA. After a 48 hour post-injection time, animals were perfused via the left ventricle of the heart with 1% paraformaldehyde, 1% glutaraldehyde in a microtubule stabilizing buffer. The brains were removed, blocked into hemi-brains and embedded in glycol methacrylate (GM). Sections were cut on a Sorvall JB-4 microtome (4 microns) and used for immunocytochemical localizations. Projection neurons were identified by incubating GM sections with antiserum to n-acetyl-WGA and visualized by our modified PAP technique. Alternate GM sections were then reacted for either substance P or methionine enkephalin. These sections were viewed stained as well as unstained to check for specificity of the PAP staining.

In order to better identify neurotransmitter and neostriatal projection neurons, alternate sections were examined by a double labeling of either projection neuron-substance P or projection neurons - methionine enkephalin. Substance P or methionine enkephalin antiserum were incubated with the GM section and identified with goat-anti-rabbit conjugated fluorescence; the sections were then re-incubated with antiserum to WGA and visualized with goat-anti-rabbit conjugated to rhodamine.

Results showed that some neostriatal projection neurons contained either substance P or methionine enkephalin. However, not all neurons that labeled as projection neurons contain substance P or methionine enkephalin as well as not all neurons labeled with substance P or methionine enkephalin were projection neurons. These data indicate that at least some neostriatal projection neurons may utilize substance P or methionine enkephalin as neurotransmitters.

(This study was supported by NIH Grant 14866.)

48.20 RESPONSES OF SUBTHALAMIC NEURONS TO INTRACELLULAR ACTIVATION, ORTHODROMIC AND ANTIDROMIC STIMULATION. S. T. Kitai, H. Kita and H. T. Chang. Department of Anatomy, Michigan State University, East Lansing, MI 48824.

Stimulation of globus pallidus (GP) has been previously shown to suppress the spontaneous firing of extracellularly recorded subthalamic (STH) units. It was also reported that this stimulation sometimes resulted in firing of STH neurons. In order to study more precise synaptic events underlying these phenomena, intracellular recording of the responses of STH neurons to GP stimulation was performed in male Sprague-Dawley rats anaesthetized with urethane (1.0-1.5 g/kg) or Ketamine (100 mg/kg). Unilateral decortication of the frontal cortex was performed in all animals in order to eliminate corticosubthalamic fibers passing through GP in which the stimulating electrodes were placed. Stimulation electrodes were also placed in the substantia nigra (SN) to study by antidromic activation and a collision technique whether single STH neurons project to both GP and SN. Square current pulses of 0.05-1.0 msec duration were used for stimulation. Bevelled glass micropipettes containing 4% horseradish peroxidase (HRP) in Tris buffer (pH 7.6) and .5 M potassium methylsulfate or 1.0 M potassium chloride were used for intracellular recording. Recorded neurons were labeled by iontophoretic application of HRP through the recording electrode.

GP and SN stimulation evoked antidromic spikes in single STH neurons with mean latency of 1.2 and 1.1 msec respectively. The mean conduction velocity of the axons of STH neurons projecting toward GP was estimated to be 2.5 m/sec and toward SN 1.4 m/sec. These differences in conduction velocities of bifurcating axons made it possible for a simultaneous arrival of STH signals in GP and SN. All STH neurons responded to stimulation of GP with short latency (mean 1.3 msec) monosynaptic IPSPs with a duration of 5-24 msec and followed by depolarizing potentials. Input resistance determined from plateau values of membrane responses to hyper- and depolarizing stimuli ranged from 9-28 MΩ (mean = 18; S.D. = 6, n = 7). Membrane time constants estimated from 1/e point of the membrane potential changes produced by weak current pulses ranged 4-9 msec (mean = 6, S.D. = 2, n = 7). Repetitive spike discharge up to 500 Hz in STH neurons was evoked by suprathreshold direct depolarizing stimuli delivered through recording electrode. Morphology of the labeled neurons indicated that all the recorded neurons were Golgi Type I neurons. Some of the axons were traced to both GP and SN. We conclude that suppression of spontaneous STH neuronal activities by GP stimulation is achieved by monosynaptic IPSPs. Excitation reported previously is most likely due to inadvertent activation of corticosubthalamic fibers coursing through GP. (Supported by NIH Grants NS 14866 to S.T.K. and NRSA 1F32NS06951 to H.T.C.)

48.21 CELLULAR COMPARTMENTS AND MOSAICS OF OPIATE RECEPTORS, AFFERENT FIBER TERMINATIONS, AND CHOLINESTERASE LEVELS IN THE NUCLEUS ACCUMBENS OF THE RAT. Sandra Moon Edley and Miles Herkenham. Lab. of Neurophysiology, NIMH, Bethesda, MD 20205.

The striatum of the rat is characterized by a mosaic fit of opiate receptors, acetylcholinesterase (AChE) levels and thalamic parafascicular nucleus projections (Herkenham and Pert, Nature, 291: 415, 1981). Using adjacent cryostat-cut sections from the same brain, we extended that analysis to the nucleus accumbens and compared cytoarchitecture, AChE enzyme histochemistry, mu-opiate receptors and the terminal distributions of thalamic or mesolimbic afferent sources.

A thionin stain of the nucleus accumbens reveals two forms of cellular compartmentalization; cell clusters consisting of 20-200 closely-packed neurons per 25 um-thick section and cell islands made visible by a thin, cell-poor rim. Sections stained for AChE reveal great enzyme heterogeneity throughout the nucleus. The medial half of the accumbens is AChE-dense while the dorsal and dorsolateral portions are AChE-poor. In addition, most of the cell clusters have low AChE levels, though some clusters are associated with very dense AChE staining. The cell islands are also characterized by low AChE levels, but both the cytoarchitectonic and AChE borders are indistinct.

Opiate receptors marked by [^{3}H]naloxone are densely and heterogeneously distributed throughout the accumbens. Dense receptor spots are in register with the cell clusters and either the AChE-poor or dense patches. The relationship between opiate receptors and AChE elsewhere is more complex. For example, in the medial accumbens, high AChE levels span a wider zone than the narrow receptor-dense stripe along the medial edge.

Injections of [^{3}H]amino acids were placed into the thalamic paratenial (Pt) nucleus or the ventral tegmental area (VTA). Strikingly heterogeneous terminal distributions were found in the nucleus accumbens. The Pt termination is punctuated by vacancies; some of these have sharp edges and correspond to boundaries of cell clusters. Another form of heterogeneity is less dramatic: larger, less-sharply defined zones of reduced terminations are in register with cell islands and AChE-poor zones. Many of these areas are also opiate-receptor dense. The terminations of VTA fibers share this latter pattern, that is, areas of low VTA termination density correspond to cell islands, low AChE levels and high opiate receptor density. Exceptions to this can be found, however.

In summary, the type of mosaic that characterizes tract terminations, opiate receptors and AChE levels in the caudate-putamen seems also to apply to the nucleus accumbens. In the accumbens, however, it is easier to find a morphological correlate of the mosaics, namely the cell clusters and islands.

48.22 CELL CLUSTERS IN THE CYTOARCHITECTONIC ORGANIZATION OF THE NUCLEUS ACCUMBENS IN THE RAT. V.B. Domesick. (SPON: C. Marotta). Mailman Research Center, McLean Hospital, Belmont, MA 02178 and Dept. of Anatomy, Harvard Medical School, Boston, MA 02115.

At present, various cytoarchitectonic, histochemical, or connectional criteria are not useful to delimit the nucleus accumbens from the remaining striatum dorsally (the caudatoputamen) and ventrally (the olfactory tubercle). In fact, the homogenous appearance of their cytoarchitecture has served to emphasize the continuity between these three striatal regions. Our purpose in studying the cytoarchitecture of the nucleus accumbens was to identify subregions that might be correlated with specific categories of afferents. Conventional histological techniques were used to prepare albino rats for light or electron microscopy. Series of sections 25 or 50 microns were stained for Nissl with cresylechtviolet. Serial semithin plastic sections were stained with toluidine blue. Ultrathin sections were en bloc stained with uranyl acetate, contrasted with lead citrate and viewed in a Siemens-Elmiskop 1A electron microscope.

In Nissl stained sections the nucleus accumbens could be characterized by an array of heterogeneous cell clusters. Varying numbers of cells were distributed in unique arrangements forming round, crescent, eliptical or strip-like shapes. Each cluster was also distinguished by the density of its cell packing. Semithin plastic sections revealed that all of the clusters were composed of subunits of two to six closely apposed cells. At the electron microscopic level the cytoplasmic membranes of diadic and triadic cell groups often showed an intervening gap of 5-10 nm. The appearance of the resulting apposition of cell membranes suggests some type of functional cell junction. The pattern of cell clustering may serve to define distinct subregions of the nucleus accumbens which may be distinguished from each other as well as from those in the remaining striatum. Cell clusters also characterize the caudatoputamen and olfactory tubercle. The entire striatum may be organized in zones defined by unique patterns of cell clusters. The possible correspondence of cell clusters to various inputs will also be explored. This work was supported by NIMH grant PO1 MH31154.

48.23 EVIDENCE FOR A PROJECTION FROM THE LATERAL PREOPTIC AREA AND SUBSTANTIA INNOMINATA TO THE MESENCEPHALIC LOCOMOTOR REGION. L.W. Swanson, G.J. Mogenson and M. Wu. The Salk Institute, La Jolla, CA 92037, and Dept. Physiol. Univ. Western Ontario, London, Ontario.

We have undertaken a series of combined neuroanatomical-electrophysiological studies to clarify the organization of neural mechanisms that mediate adaptive, goal-oriented behavioral responses commonly associated with the hypothalamus. One of the most important components of such responses must involve an interaction between the hypothalamus and somatomotor control systems for locomotion, and for head and eye movements during foraging behavior. Considerable evidence (Brain Res. Rev. 3:1, 1981) suggests that locomotor activity can be modulated by a multisynaptic pathway that first involves projections from the hypothalamus to the ventral tegmental area, and second involves projections from the latter to the nucleus accumbens.

In a previous study, we (Mogenson, Swanson and Wu; J. Neurosci., in press) described the topographical organization of projections from the nucleus accumbens to the ventral globus pallidus and a subpallidal region that includes parts of the substantia innominata, lateral preoptic area and lateral hypothalamic area, and showed that electrical stimulation of the nucleus accumbens changes the spontaneous discharge rate of many neurons in the ventral pallidium and subpallidal region.

The present series of experiments was designed to determine whether cells in the subpallidal region of the rat project directly to dorsal parts of the midbrain reticular formation that Shik and Grillner have called "the mesencephalic locomotor region", which is centered in the pedunculo-pontine (PPN) and cuneiform nuclei in the cat. First, a series of anatomical studies with axonally transported markers was carried out. Autoradiographic experiments with injections of ^{3}H-amino acids confined to the subpallidal region demonstrated a pathway to the PPN and adjacent parts of the central gray, and retrograde tracer experiments with injections of true blue in the PPN confirmed that many cells in the substantia innominata, lateral preoptic area, and lateral hypothalamic area (as well as some cells in the n. accumbens itself) project to the PPN. And second, a series of electrophysiological experiments was carried out to determine whether the spontaneous discharge rate of individual neurons in and around the PPN of urethane anesthetized rats was influenced by single-pulse stimulation of the substantia innominata and lateral preoptic area. It was found that the spontaneous discharge rate of 42 out of 47 neurons tested was changed, that the most frequent response was suppression of the discharge rate, and that the onset latency of most responses was between 2.5-5 msec.

These results suggest that some cells in the substantia innominata and lateral preoptic area may be directly involved in the modulation of locomotor responses, although further work is needed to determine the role of the dorsal midbrain tegmentum in somatomotor control mechanisms.

49.1 CATECHOLAMINE AND INDOLEAMINE CONCENTRATIONS IN THE DEVELOPING RAT HYPOTHALAMUS. A.G. Watts* and H.F. Stanley* (SPON: G. Fink). MRC Brain Metabolism Unit, Department of Pharmacology, 1 George Square, Edinburgh, U.K.

The effect of sexual differentiation of the brain upon central neurotransmitter systems is far from clear. Here we report the changes in concentrations of catecholamines and indoleamines throughout development in neuronal areas which have been shown to have sex dependent morphological differences. The concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were simultaneously determined (using HPLC with electrochemical detection) in the preoptic area and hypothalamus of male and female rats at 36 hr and 4, 12, 20, 40 and 80 days of age. No detectable differences in the concentrations of any of the four compounds were found between the sexes at any age. Concentrations of each compound increased slowly over the first 12 d after birth; however, between day 12 and day 20 a rapid increase in the concentration of all compounds was found. At day 20, 5-HT and DOPAC had reached the concentrations found in adults while 5-HIAA was at its greatest concentration. DA concentrations increased steadily throughout development, maximum concentrations being reached in adults. The molar ratio of 5-HIAA to 5-HT is thought to be an indicator of the turnover rate of 5-HT (Tozer, T.N., Neff, N.H. and Brodie, B.B., J. Pharm. & Exp. Ther. 153, 177, 1966). In the present study the ratio reached a maximum at 4 d of age with lowest values being found in adults. No significant differences between the sexes were found during development; however, small but significant differences were found at 40 d (males 0.72 $\pm$ 0.02, females 0.67 $\pm$ 0.01; $p < 0.05$) and adults (males 0.43 $\pm$ 0.01, females 0.49 $\pm$ 0.01; $p < 0.001$). Currently, experiments are being carried out with the aid of the tryptophan decarboxylase inhibitor, m-hydroxybenxylhydrazine, to determine whether the ratio differences found between animals at 4 d and adults were due to either differences in turnover, differences in MAO activity or the mechanisms of acid metabolite excretion.

These results show that any sexually dependent differences in dopaminergic or serotonergic systems are not reflected by differences in total content. However, the results do suggest that the rates of development of dopaminergic and serotonergic systems differ from one another.

49.2 CARBOHYDRATE COMPONENTS DURING CEREBELLAR DEVELOPMENT OF NORMAL AND MUTANT MICE IN VIVO IN VITRO, AND IN OCULO. W. Wille*, Institute for Genetics, University of Cologne, Fed. Rep. Germany (SPON: E. Trenkner)

De novo synthesis, changes in composition, and degradation of carbohydrate (CHO)-containing molecules are developmentally dependent events in the cerebellum. Some of the involved glycosidases express age-dependent differences in specific activity during postnatal development of the cerebellum and differ significantly in the corresponding tissue of the neurological mutant staggerer (sg)(Wille & Trenkner, J. Neurochem., 37:443, 1981; Wille et al.,in press). Indirect evidence for differences in cerebellar surface CHO depending on age and genome has been described earlier (Hatten & Messer, Nature, 276:504, 1978; Trenkner, Nature, 277:566, 1979).

One phenotype of the staggerer (sg/sg) mutation (degeneration of cerebellar internal granule cells) is also expressed in transplants of embryonic sg/sg-cerebellum "anlage" grafted, grown, and differentiated in eyes of +/+ recipient mice (Wille et al., submitted). This proves the intrinsic quality of the cerebellar mutation in staggerer. Besides the cerebellum in vivo and the microwell cell cultures (Trenkner & Sidman, J.Cell Biol., 75:915, 1977) the transplants provide an excellent model system of intermediate complexity for neuronal histogenesis offering the opportunity of manipulating the CHO-metabolism. Effects of exogenous interference in defined steps of development-dependent CHO-pathways can be analyzed histologically, histochemically, and biochemically.

Cerebellum transplants (grafted at embryonic day 11 from wildtype donors) differentiate in oculo into cerebellum-like tissues containing the requisite neuronal and glial cell types (Hoffer et al., Brain Res. 79:165, 1974). Inhibition of glycoprotein synthesis with tunicamycin applied to the grafts by intraocular injection 8 to 15 days after grafting ("P0 - P7") cause selective granule cell death as analyzed in 45 days old transplants. Injections at "P10" or later do not affect the survival of cerebellar granule cells.

Experiments with specific inhibitors of the CHO-metabolism and their effects on histogenesis and the composition of glycoproteins and gangliosides during cerebellar development in vivo, in vitro, and in oculo are presented.

49.3 IN VITRO TRANSLATION OF NEURON-SPECIFIC PHOSPHOPROTEINS: DEVELOPMENTAL STUDIES AND MESSENGER RNA PURIFICATION. S. Kanazir*, R.M. Lewis*, W.C. Wallace*, L.J. DeGennaro*† and P. Greengard. Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510, U.S.A. and †Max Planck Institute for Psychiatry, Dept. Neurochemistry, 8033 Martinsried, Federal Republic of Germany

In an effort to correlate synaptogenesis with the appearance of specific neuronal phosphoproteins, we have developed an in vitro translation assay of brain polypeptides. Protein I, a synaptic vesicle-associated protein which is localized specifically in presynaptic nerve terminals, is phosphorylated by Ca^{2+}/calmodulin-dependent and cAMP-dependent protein kinases. Protein III, also found in nerve terminals, is phosphorylated by cAMP-dependent protein kinase. We have now studied the relative levels of synthesis of Protein I and Protein III in developing postnatal rats. Polysomes were isolated from rat brain at various ages and translated in a rabbit reticulocyte lysate. The newly-synthesized proteins were immunoprecipitated with specific antibodies and measured. The relative level of synthesis of Protein I increased from birth (0.05% of total ^{35}S-methionine incorporated into protein) to a maximum level at day 14 (0.33%), then decreased to an adult level of 0.15%. The changes in the synthesis of Protein III were similar to those observed for Protein I. We have also studied the synthesis of G-substrate, a Purkinje cell-specific protein phosphorylated by cGMP-dependent protein kinase. This protein was detected as early as day 8 in postnatal rat cerebella.

Messenger RNA's (mRNA) for Protein I and Protein III were partially purified by specific immunoprecipitation of polysomes followed by oligodT-cellulose affinity chromatography (suggesting that both mRNA's are polyadenylated). We have also partially purified the mRNA for a cytosolic protein (32K) phosphorylated by a cAMP-dependent protein kinase in dopaminoceptive neurons. We are continuing studies of neuronal biosynthesis in relation to synaptogenesis using recombinant DNA techniques.

49.4 DEVELOPMENT OF GABAergic NEURONS IN PRIMARY CULTURES OF RAT RETINA. I. Pech* and P.V. Sarthy. Depts. of Ophthalmology, Physiology and Biophysics, University of Washington, School of Medicine, Seattle WA 98195.

We are interested in the development of neurotransmitter systems and synapse formation in the vertebrate retina. In order to study these problems in vitro, we have developed conditions for maintaining rat retinal neurons in culture. Retinas were dissected from 16 day old rat fetuses and dissociated after trypsinization. The resulting cell suspension was plated onto polyornithine coated plates in Leibovitz's L-15 medium supplemented with 10% horse serum and maintained in an air atmosphere. Under these conditions, monolayer cultures of retinal neurons could be maintained for up to one month. That these cells were neurons was confirmed by the presence of tetanus toxin receptors on their surface. Two distinguishing features of these cultures were: (i) few non-neuronal (flat) cells were present, and (ii) the neurons did not aggregate but remained as individual cells so that their survival and process formation could be readily assayed by cell counting.

In order to establish the presence of GABAergic neurons, the synthesis, release and uptake of GABA were studied in these cultures. GABA synthesis was demonstrated by incubating cells with ^{3}H-glutamate and measuring the formation of ^{3}H-GABA. The presence of glutamic acid decarboxylase (GAD), an enzyme involved in GABA synthesis, was also confirmed by a radiometric assay. Cultures preloaded with ^{3}H-GABA were shown to release ^{3}H-GABA upon depolarization with 48 mM K^+. Further, this release was suppressed in the presence of 5 mM Co^{2+}. GABA uptake was established by two different methods. Light microscopic autoradiography indicated that virtually all cells took up ^{3}H-GABA and that this uptake was almost completely blocked by 1 mM GABA, DABA and Nipecotic acid, and only partially by β-alanine. Biochemical uptake studies showed the existence of a high affinity GABA uptake system in these cells.

Development of the above parameters was studied in cultures up to 3 weeks. ^{3}H-GABA synthesis was first detected at 4 days in vitro (d.i.v.) and increased thereafter, up to 2 weeks in culture. GAD activity showed a parallel increase with time. A small amount of GABA release was noted as early as 2 d.i.v.. GABA was taken up by a small fraction of the cells as early as 1 d.i.v.. With longer time periods in culture, an increasing number of cells took up ^{3}H-GABA, and the intensity of labeling was also enhanced.

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49.5 MECHANISMS REGULATING THE BIOCHEMICAL DEVELOPMENT OF THE LOCUS COERULEUS (L.C.) IN CULTURE. C.F. Dreyfus, K.A. Markey, M. Goldstein and I.B. Black. Div. of Developmental Neurology, Cornell Med. Coll., 515 E. 71st St. New York, N.Y. 10021 & Dept. of Psychiatry, New York University Medical Center, New York, N.Y. 10016.

In previous work we have found that a number of catecholaminergic phenotypic characters develop during growth of the embryonic mouse l.c. in explant culture. In the present studies we have examined underlying ontogenetic mechanisms.

Explantation of the l.c. on embryonic day 12 (E-12), prior to normal expression of tyrosine hydroxylase (T-OH) or dopamine-β-hydroxylase(DBH), noradrenergic biosynthetic enzymes, results in the de novo expression of these phenotypic characters after 7 days. Moreover, explantation of the E-18 l.c., after early expression of noradrenergic traits has occurred in vivo, results in a marked quantitative increase in T-OH activity, to levels greater than that attained in E-12 cultures.

To define mechanisms of development in vitro, we focused on the E-18 cultures. Immunotitration studies with a specific antiserum revealed that the increase in T-OH activity in these cultures was entirely attributable to accumulation of increased enzyme molecules. Further, the increase in T-OH activity was inhibited by cycloheximide, camptothecin and actinomycin D, suggesting that protein- and RNA-synthesis are required for the elevation of T-OH in vitro. Finally, immunotitration revealed that the higher levels of T-OH activity seen in E-18 cultures when they are compared to E-12 cultures was due to increased numbers of enzyme molecules. Morphometric studies indicated that this difference in T-OH protein was not attributable to differences in l.c. neuron numbers in the E-12 and E-18 cultures.

We conclude that development in the brain in situ is not necessary for initial phenotypic expression or for subsequent increases in T-OH and DBH. Our studies suggest that l.c. neurons are capable of actively elaborating T-OH molecules in the tissue culture setting in the absence of normal afferent and efferent connections. (Supported by NSF Grant BNS 8024081).

49.6 DEVELOPMENT OF CATECHOLAMINES AND ADRENERGIC RECEPTORS IN THE RAT SPINAL CORD. Leonard L. Ross, Arlene Pylypiw*, and Walter Chmelewski.* Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

The sympathetic preganglion neurons in the spinal cord receive a substantial descending catecholaminergic (CA) input from supraspinal levels. In the chick, we have reported that the descending developing axons contain catecholamines and that they establish their first synaptic contacts with spinal cord preganglionic neurons at 10-12 days in ovo. Adrenergic receptors appear 4 days later. The present study was undertaken in order to characterize the CA development sequence in the rat and relate it to parallel studies of peripheral innervation.

α- and β-adrenergic receptors were measured in homogenates of thoracolumbar spinal cord by the specific binding of ^{3}H-prazocine or ^{3}H dihydroalprenolol (DHA), respectively. Receptor density (B max) and the apparent dissociation constant (Kd) for ligand binding were calculated by Scatchard analysis. Levels of adrenaline (A) noradrenaline (NA) and dopamine (DA) were determined using high performance liquid chromatography with electrochemical detection.

The presence of α-receptors was first noted at E16 (B max = 9 fmoles/mg protein). From E16 to E22 the number of receptors increases to 116 fmoles/mg protein. At birth, receptor density falls to 80 fmoles/mg protein, rises over the next 2 weeks to the highest prenatal level and gradually falls to the adult level of 50 fmoles/mg protein. β-adrenergic receptors are not detectable until E18. They increase in density slowly pre- and postnatally to a peak of 120 fmoles/mg protein at age 15 days. This is followed by an abrupt fall to the adult level of 70 fmoles/mg protein. The affinities of both receptors did not change during development.

NA is first detectable at E16 at a concentration of 60 pg/mg. The concentration rises gradually to a peak of 410 pg/mg at 20 days postnatally and falls to an adult level of 210 pg/mg. DA is not detected until 1 day postnatally at a level of 15 pg/mg. It follows the same time course, rising to 110 pg/mg at 20 days and then falling to the adult level of 50 pg/mg. A is not detectable at any age.

Of interest are the presence of NA 2 days before the appearance of β-adrenergic receptor and the dramatic increase in both transmitter and receptor during the first 2 weeks of postnatal life. This time is also the period of the most intense peripheral autonomic synapse formation. The relationship between these two events is under investigation.

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49.7 ONTOGENY OF SUBSTANCE P-LIKE AND ACETYLCHOLINESTERASE STAINING IN THE DEVELOPING AVIAN CILIARY GANGLION. J.T. Erichsen, B.M. Davis and H.J. Karten. Dept. of Neurobiology & Behavior, SUNY at Stony Brook, New York 11794.

Substance P-like immunoreactivity (SPLI) and enkephalin-like immunoreactivity (ELI) are found in the preganglionic terminal endings of the avian ciliary ganglion (Erichsen et al., J. Neurosci., in press). The present report describes the ontogeny of SPLI and acetylcholinesterase (AChE) staining in the chick ciliary ganglion and the relationship between the immunoreactive endings and presumptive cholinergic neurons during development.

SPLI first appears in the ciliary ganglion at Stage 26 (St 26). From St 26 to St 35, SPLI staining is diffuse and weblike in appearance and shows no obvious morphological specializations around individual neurons. At St 37, pale fluorescent SPLI rings can be found around a small number of neurons. At St 40, there are many more pale immunoreactive endings, as well as several intensely labeled SPLI endings. At hatching, the intensity of SPLI is greater in an increasing number of preganglionic endings, a trend that continues through 7 days post-hatching. Although consistently less intense than ELI in the same endings, SPLI appears to increase in intensity as ELI decreases. Recent RIA studies by White et al. (Neurosci. Abs., 1982) have confirmed this apparent developmental trend in the intensity of SPLI.

AChE histochemistry revealed that most, if not all, postganglionic neurons in the ciliary ganglion contain AChE at St 30 and continue to show intense staining throughout subsequent development. Using combined immunohistochemical and AChE staining techniques, SPLI (as well as ELI) was demonstrated in terminals on these presumptive cholinergic cells.

SPLI usually co-occurs with ELI in terminal endings in both the chick and pigeon (Erichsen et al., Nature, 1982) ciliary ganglion. Yet the ontogeny of SPLI is notably different from that of ELI: SPLI is minimal until St 37 and then increases progressively until after hatching; ELI reaches a maximum at St 37 and then declines slightly. Both SPLI and ELI are present in preganglionic endings before synapses in the ciliary ganglion begin to function (Landmesser & Pilar, 1972). Therefore, in addition to their putative role in neurotransmission, substance P and enkephalin may also be involved in the regulation of synaptogenesis. Supported by EY07039 to J.T.E. and EY02146 to H.J.K.

49.8 THE FUNCTIONAL ONTOGENY OF DOPAMINE AUTORECEPTORS IN THE NUCLEUS ACCUMBENS, OLFACTORY TUBERCLES AND STRIATUM IN THE RAT. F. Scalzo* and L.P. Spear, Dept. of Psychology, SUNY-Binghamton, Binghamton, NY 13901.

The regulation of dopamine (DA) synthesis and release in nigrostriatal and mesolimbic brain regions is thought to be partially mediated by presynaptic DA autoreceptors. The interruption of impulse flow along DA afferents by lesion or pharmacological blockade with drugs such as gamma-butyrolactone (GBL) leads to an activation of tyrosine hydroxylase activity and a subsequent increase in DA in the terminal regions of these pathways. This effect is attenuated by pretreatment with a DA agonist such as apomorphine.

Shalaby and Spear (1981), on the basis of psychopharmacological studies with apomorphine, have suggested that DA presynaptic receptors mature relatively late in ontogeny when compared with dopamine postsynaptic receptors. In a subsequent neuropharmacological investigation, Shalaby, Dendel and Spear (1981) refined this hypothesis to suggest that autoreceptors in the nigrostriatal and mesolimbic DA systems may mature at different ages. They found a consistent apomorphine attenuation of a GBL-induced increase in DA by day 14 in the striatum, but not until 35 days postnatally in the olfactory tubercles (OT).

In the present report, the development of presynaptic DA autoreceptors was neuropharmacologically investigated in the striatum, OT and nucleus accumbens (NAC). One-hundred forty-eight Sprague-Dawley albino rats were used. Animals 14, 21, 28, 35 and 49 days postnatally were injected with 750 mg/kg GBL alone, 1 mg/kg apomorphine alone, a combination of apomorphine and GBL, or 1% ascorbate and .9% saline. Forty minutes later, animals were sacrificed, their brains removed and striata, OT and NAC dissected and assayed for DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). In addition, determinations were made in the striatum of 7-day old animals under all drug conditions.

GBL significantly increased DA levels in the striatum at 21, 28, 35 and 49 days of age. This increase was consistently attenuated by apomorphine pretreatment beginning at 28 days. In the olfactory tubercles, while GBL significantly increased DA levels at 28, 35 and 49 days, this increase was attenuated only at 35 days. In NAC, GBL had no effect on DA levels. Measurement of dopamine metabolites (DOPAC and HVA) revealed an apparent alteration in DA utilization with age in striatum. These results suggest that DA presynaptic autoreceptors mature later in OT than in striatum while, in the NAC, presynaptic DA receptors appear to be less sensitive to the effects of GBL.

49.9 QUANTITATION OF THE DEVELOPMENTAL SENSITIVITY OF NEONATAL RAT EEG TO THE CONVULSIVE EFFECTS OF GAMMA-HYDROXYBUTYRIC ACID. L.J. Bearden, O.C. Snead and H.I. Stephens*. Dept. of Pediatrics and Neurosciences Program, Univ. Alabama in Birmingham, Birmingham, AL 35294.

The gamma-hydroxybutyric acid (GHB) model of petit mal seizures has several intriguing characteristics. Perhaps the most salient of these is that GHB produces electrical and behavioral effects which are very much like those seen during absence seizures. Moreover, these electrical and behavioral effects of GHB are blocked by the anticonvulsants which are used successfully for the clinical management of petit mal epilepsy. There also appear to be developmentally related aspects of the endogenous supply of GHB. For example it has recently been observed that the concentration of GHB in rat brain decreases slowly from 14 days prenatal to 13 days postnatal, then falls dramatically between 13 and 20 days postnatal (Snead and Morley, Dev. Brain Res. 1:579-589, 1981). The relation between this decrease in GHB concentration and the development of the brain is not known.

The developmental aspects of the EEG are well characterized in primates. However, the manner in which the EEG changes with age in many laboratory animals is not well defined (Epstein, Developmental Psychobiology 13:629-631, 1980). Such information is of obvious importance to experimental work. But a clear understanding of these changes is essential for laboratory animal studies of age related disorders such as petit mal epilepsy. Recent experiments in our laboratory have focused on quantitating the sensitivity of the developing rat brain to the convulsive electrical effects of GHB.

Male Sprague-Dawley rats (age 24 hrs-4 wks) from our own breeding colony were used in these experiments. Animals were implanted with epoxylite insulated tungsten macroelectrodes in the cortex, hippocampus, caudate and amygdala under halothane anesthesia and allowed to recover from surgery. Rats were given various doses of sodium gamma-hydroxybutyrate i.p. Bipolar EEG was recorded from each electrode on a polygraph (Grass, Model 78) and on FM tape (Ampex, FR 1300). The EEG was analyzed visually and by means of a Fast Fourier Transform algorithm which was coded in Fortran to run on a minicomputer (Data General, Nova 2). Power spectral density plots of the results of this type analysis were generated as a compressed spectral array on a plotter (Cal Comp 1039) under computer control (Honeywell/Xerox 560). The sensitivity of rats to the electrical effects of GHB increased with increasing age. It was also observed that at the onset of the electrical effects of GHB, the convulsive activity appeared discretely in neonatal animals as compared to the gradual appearance seen in adult animals. These results indicate a developmental sensitivity of the rat brain to the convulsive electrical effects of GHB.

49.10 MULTIPLE IMMUNOREACTIVE FORMS OF MET- AND LEU-ENKEPHALIN IN FETAL AND NEONATAL RAT BRAIN AND IN RAT GUT. Barbara L. Silva*, June L. Dahl, Miles L. Epstein† and Iris Lindberg*†† (SPON: Hanna Sobkowicz). Departments of Pharmacology and †Anatomy, University of Wisconsin Medical School, Madison, WI 53706, and ††Laboratory of Preclinical Pharmacology, NIMH, St. Elizabeth's Hospital, Washington, D.C. 20032.

We have used carboxyl-specific antisera to study the development of met- and leu-enkephalin in rat brain and gut. Although both peptides were detected early in ontogeny (at 13-16 days of gestation), the radioimmunoassay data showed different developmental profiles for the two peptides and an apparent 4-fold increase in the ratio of met- to leu-enkephalin from fetal life to adulthood. However, fractionation of HCl extracts of fetal and neonatal brain tissue by HPLC revealed the presence of immunoreactive forms other than met- and leu-enkephalin. For example, in extracts of 16-day fetal and 1-day neonatal brain, there were two peaks of leu-enkephalin immunoreactivity. One emerged at the position of leu-enkephalin, the other eluted with about one-third the retention time. There were four peaks of met-enkephalin immunoreactivity in extracts of fetal brain, one with the retention time characteristic of met-enkephalin, the others with shorter retention times. In contrast, all of the met-immunoreactivity in adult brain eluted with the retention time characteristic of authentic met-enkephalin and all of the leu-enkephalin immunoreactivity eluted in the position of authentic leu-enkephalin. Multiple immunoreactive forms of met- and leu-enkephalin were found in extracts of both fetal and adult gut tissues. Characterization of these various immunoreactive forms is in progress. Preliminary results suggest that the leu-enkephalin-like peptide present in embryonic brain and in gut is a low molecular weight, hydrophilic molecule which is converted to leu-enkephalin in the presence of TFA. We speculate that it may be a phosphorylated or sulfated form of leu-enkephalin. It is interesting in this regard tha an O-sulfated leu-enkephalin has very recently been reported to be present in rat brain [Unsworth et al. (1982) Nature 295, 519]. Supported in part by funds provided by the Medical School Research Committee (JLD) and by NIH grant NS16280 (MLE).

49.11 CULTURED EMBRYONIC CEREBELLAR CELLS ACCUMULATE ^{3}H GABA. S. Roffler-Tarlov and M. E. Hatten, Dept. of Neurology, Tufts University School of Medicine, Boston, MA 02111 and Dept. of Pharmacology, New York University Medical Center, New York, NY 10016.

The autoradiographic localization of ^{3}H GABA captured by high affinity uptake systems present in gamma amino butyric acid (GABA) utilizing neurons has been a useful technique for identifying GABA neurons in mature brain. We have applied the technique for study of the development and interaction of cells disassociated from developing mouse cerebellum on embryonic day (E) 13. At E 13 the mouse cerebellum consists of proliferating young neurons from subventricular and ventricular zones, post mitotic neurons which have begun to migrate in the intermediate zone, proliferating young granule neurons from the emerging external granular layer and immature glia. The major types of neuronal precursors are those of granule cells, Golgi II cells, Purkinje cells and deep cerebellar neurons. Several lines of evidence point to GABA as the transmitter for both Golgi II cells and Purkinje cells but not of granule cells or the deep nuclear cells. Examination of autoradiograms of E 13 cerebellar cells exposed to ^{3}H GABA (5.8×10^{-7} M) for 30 min, after one day in monolayer culture showed that approximately 30% of the cell bodies accumulated ^{3}H GABA. After 3-5 days in vitro heavy accumulation was also observed in the processes of these cells. The cells are able to accumulate ^{3}H GABA nearly a week before synapses are made in culture.

Two lines of evidence suggest that the majority of cells which accumulate ^{3}H GABA are neurons. First, total uptake of ^{3}H GABA was reduced 47% by 100 µM diamino butyric acid (DABA) an antagonist of the neuronal uptake of GABA and 10% by the glial uptake blocker beta alanine (100 µM). The effect of DABA on uptake could be visualized in autoradiograms. Second, double labeling of cultures with glial filament antibody and ^{3}H GABA showed that the majority of cells which accumulate GABA are neurons.

These experiments show that a specific neurotransmitter characteristic distinguishes between groups of cerebellar neurons before migration and the establishment of synapses has taken place.

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49.12 DEVELOPMENTAL CHANGES IN THE LEVELS OF N,N-DIMETHYLTRYPTAMINE AND RELATED INDOLEALKYLAMINES IN RAT BRAIN. J.M. Beaton and P.E. Morris*. Neurosciences Program and Dept. of Psychiatry, Univ. of Ala. in Birmingham, Univ. Station, Birmingham, Al 35294.

In the last decade the hallucinogen N,N-dimethyltryptamine (DMT) has been identified as a normal constituent of human urine, blood and cerebrospinal fluid. It has subsequently been characterized as a putative neurotransmitter or neuromodulatory substance in rat brain. Using an isotopic dilution gas chromatographic/mass spectrometric assay for the simultaneous quantification of tryptamine (T), DMT, O-methyl-bufotenin (OMB), tetrahydro-beta-carboline (THBC) and 6-methoxy-tetrahydro-beta-carboline (6-MeOTHBC), the brain levels of these compounds were measured in rats of various ages (1-60 days old). The subjects were mixed sex, Sprague-Dawley rats bred in our animal facilities. The time of birth of each litter was noted (within a 3 hour time range). Groups of rat pups were then sacrificed by decapitation at age 24 hours, 72 hours and 7, 10, 14, 17, 21, 24, 28, 31, 35, 40, and 60 days. For each analysis sample approximately 2 g of brain tissue was used, necessitating the pooling of several brains for the younger animals. Five samples were analyzed for each age tested. All subjects were group-housed in a 12 hour light/dark cycle. After sacrifice the brains were frozen in liquid nitrogen and stored at -70^0 for later analysis. The analyses were carried out using deuterated internal standards as previously reported (Biochem. Pharmacol. 29, 1049, 1980) and the compounds were derivatized with heptafluorobutyryl imidazole. The endogenous compounds were quantified against the internal deuterated standard.

Varying levels of all compounds were identified at most age ranges tested. As has been previously reported the levels of the compounds were very low in the older rats (35 days and above), however, the levels of DMT and OMB were relatively high in the younger rats, peaking at age 22 days. The median values for DMT ranged from 1.3 ng/g wet weight in 35 day old rats (it was not detected in 60 day old rats), to 11.7 ng/g wet weight in 22 day old rats. The corresponding values for OMB, THBC and 6-MeOTHBC were 3.4 to 31.4, 5.3 to 14.2 and 9.6 to 25.3 ng/g wet weight, respectively. There was considerable variation between samples, which may have been due to a male-female sex difference. (Currently, the levels of these compounds are being measured separately in male and female rats at several ages.) Although the roles for DMT and the other compounds in brain has not been determined, it is of interest to note that the levels of these compounds does vary in the developing rat brain.

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49.13 PLASMINOGEN ACTIVATOR PRODUCTION AND GLIAL CELL DIVISION: PARALLEL EVENTS IN THE DEVELOPING CHICK SPINAL CORD. N. Kalderon. The Rockefeller University, New York, NY 10021.

The pair of proteases, plasminogen activator and its reaction product plasmin, has been implicated in several tissue remodelling processes. It was previously reported (Kalderon, PNAS 1979. 76, 5992-5996) that chick embryonic spinal cord cells produce plasminogen activator, and that this plasmin-generating system is essential for the tissue morphogenesis to assume a normal pattern in vitro. In the present study, the production of plasminogen activator by embryonic spinal cord tissue as a function of spinal cord development was determined on chick embryonic spinal cords, starting from day 4 of incubation until hatching. Spinal cords were collected from each embryonic day (4-20) and solubilized in 0.2% Triton X-100. The lysates were assayed on [^{125}I]fibrin-coated plates supplemented with purified plasminogen. It was found that the developing spinal cord produces plasminogen activator. This production is markedly enhanced during three stages: on days 8, 11, and 14-16. The fact that the level of production of plasminogen activator varies during the different stages of development implies that the production of this enzyme is switched on and off during the differentiation process. Several gross tissue remodelling processes are taking place within the chick embryonic spinal cord, e.g., cell death of motoneurons (massive degeneration between days 6-9; Chu-Wang & Oppenheim 1978. J. Comp. Neur. 177, 59-86), glial cell division (starting from day 8 on; Fujita 1965. J. Comp. Neur. 124, 51-60). In an attempt to correlate the peaks of plasminogen activator production with these tissue remodelling processes the content of DNA per protein of the developing spinal cord was determined, on each day, starting from day five until day seventeen of incubation. The ratio of DNA/protein peaks during several stages of development, specifically on days 5, 8, 11 and 14. These peaks on days 8, 11, and 14 indicate bursts of cell division which are probably due to glial cell division since the neuroblasts only divide during days 3-8. It seems that glial cell division and the production of plasminogen activator occur at the same time. The two events are probably closely related as plasminogen was found to be mitogenic for glial cells in vitro. (Supported by grants from NIH NS17169 and Muscular Dystrophy Association)

49.14 PHARMACOLOGIC ONTOGENY OF SEROTONERGIC AND DOPAMINERGIC NEURONS IN THE RAT. W.H. Lyness. Department of Pharmacology and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, Texas 79430.

Neonatal rats administered amphetamine-analogs or neuroleptics, it is known, behave comparably to their adult counterparts, i.e., hyperactivity-stereotypy or sedation. This study examined tissue punches of striatum, nucleus accumbens (NAS), frontal cortex (FC), and the olfactory tubercles for the neurochemical responses to dopamine (DA) agonists and antagonists in rats 5 days to 180 days old. The DA concentrations of 5 day old rats were lower in all areas and increased with age. The NAS appeared the most mature in regard to DA content and levels of the two DA metabolites DOPAC and HVA. Extensive dose response curves with haloperidol and clozapine show less sensitivity to drug-induced DOPAC and HVA increases in 5 day old rats. The magnitude of drug-induced metabolite increases approaches the adult response by 15 days old. Conversely, DA agonists apomorphine and bromocriptine, which characteristically lower DOPAC-HVA concentrations, did so comparably in all brain regions except FC of 5 day olds. These results might suggest a maximal DA synthesis and release which cannnot be enhanced easily by DA-antagonists but can be attentuated by DA agonists. Studies using γ-butyrolactone (GBL) show increases in DA content of all brain areas except FC as early as 5 days old suggesting DA presynaptic autoreceptors are functional.

Studies of neonatal cortical 5-HT neuronal systems indicates a lower (20% of adult values) absolute level of 5-HT but near adult levels of its metabolite 5-HIAA (80% of adult values). Brain levels of tryptophan (TRY) were 3 times those of adults at 2 days old and rapidly declined with age. TRY loading (50 mg/kg i.p.) increased brain TRY in rats of all ages but failed to increase 5-HT concentrations in 2 and 5 day old rats. Treatment of animals with NSD-1015 results in linear accumulations of 5-hydroxytryptophan with time in rats of all ages but indicated higher rates of 5-HT synthesis in neonatal rats. While TRY loading produced further increases in brain TRY in 2 and 5 day old rats over untreated animals, the failure to increase 5-HT content might be due to the already maximal synthesis rate in neonatal rats. Increased synthesis of neurotransmitter in neonates appears to be a common factor in both the DA and 5-HT neuronal systems although the responsible mechanisms may be different.

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49.15 DEVELOPMENT OF CENTRAL GABA MECHANISMS: NEUROCHEMICAL AND FUNCTIONAL ASPECTS. T. Hedner*, J. Hedner*, K. Iversen*, J.Jonason* and P. Lundborg*. (SPON. N. Bass) Dept of Pharmacology, Univ. of Göteborg, and Dept of Clinical Pharmacology and Pediatrics, Sahlgren´s and East Hospitals, Göteborg, Sweden.

Previous ontogenetic studies have indicated that GABA mechanisms may be relatively mature during early stages of development. In order to further study these mechanisms we have investigated 1) the postnatal regional development of GABA levels as well as accumulation after GABA-T inhibition, 2) effects of experimental asphyxia on GABA levels and accumulation in 4 days old rats, 3) CSF GABA concentrations in human neonates, 4) respiratory effects after GABA administration to preterm rabbits.

Rats of various postnatal ages (1-60 days) were used in the experimental studies.

3-mercaptopropionic acid was given immediately before sacrifice and brain dissection to all animals. In the human, CSF samples were collected on ice and immediately deepfrozen (-70°C) until analysis. GABA was assayed by a fluorimetric method. Respiratory studies were performed on anesthetized (halothane in O_2) preterm (29 days gestation) rabbits in a whole body plethysmograph after intraperitonal injections of GABA, 750 mg/kg.

During the immediate postnatal period in the rat regional GABA levels were relatively high. Until 7 days of age a general decrease was seen in most regions (striatum, midbrain, brainstem, cerebellum, upper and lower spinal cord). Increases were then seen in all regions except for the spinal cord where GABA levels continuously decreased until adult age. GABA accumulation after aminooxyacetic acid (AOAA) was more marked at older ages. In 4 days old rats 60 min of 6% hypoxia induced an increase in GABA levels during and shortly after the hypoxic insult. CSF GABA concentrations in the human infants ranged between 8-45 nmoles/ml which is approximately 20-100 times the concentrations found in adults. Infants with perinatal asphyxia demonstrated increased CSF GABA concentrations compared to respective controls. GABA administration to preterm rabbits depressed both basal respiration and CO_2 induced respiratory stimulation. The decrease in basal pulmonary ventilation was mainly due to a decrease in tidal volume, as respiratory frequency was not significantly altered.

From the present studies we conclude that central GABA mechanisms are relatively biochemically as well as functionally mature during early postnatal age. Neonatal asphyxia causes an increase in central GABA activity which may be an etiological factor for the respiratory depression seen in this and several other neonatal disorders.

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49.16 COMPARISON OF CSF MONOAMINE METABOLITE AND PRECURSOR MEASUREMENTS IN THE RAT PUP AND IN HUMAN CHILDREN. B.A. Shaywitz, G.M. Anderson*, J.G. Young and D.J. Cohen*. Labs. Devel. Psychobiol. & Neurochem., Yale Univ. Sch. of Med., New Haven, CT 06510.

The monoamine metabolites homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-5-hydroxyphenylethyleneglycol (MHPG) have been extensively measured in human cerebrospinal fluid (CSF) in an attempt to estimate central turnover of the parent amines (dopamine DA, serotonin 5-HT, and norepinephrine NE respectively). Although correlations have been observed between brain and CSF metabolite levels in both humans and rats it is not clear to what extent changes in turnover and metabolite levels in different brain areas will be reflected in CSF levels. The measurements made in CSF from children are further confounded by developmental aspects. We have previously reported preliminary studies centered around measurements made in CSF obtained from the developing rat pup. We have now further characterized the ontogeny of the compounds, their interrelationships, and the effects of alterations in brain neurochemistry on CSF levels. We are particularly interested in the effects of pharmacological agents which might be used to treat neuropsychiatric disorders of childhood. Thus, we have examined the effects of methylphenidate, amphetamine, and fenfluramine on precursor (tryptophan and tyrosine) neurotransmitters (5-HT, NE, DA) and metabolite levels (5-HIAA, HVA, and indoleacetic acid) in whole brain, brain areas and cisternal CSF of the developing rat pup. The age dependence of the alterations will be presented and the results compared to our and others' findings in children.

49.17 SUBCELLULAR DISTRIBUTION OF POLYAMINE ACETYLATION IN THE DEVELOPING MOUSE BRAIN. J.G. Ortiz and E. Giacobini. Lab. of Neuropsychopharmacology, Dept. of Biobehav. Sci., Univ. of Conn., Storrs, CT 06268.

Acetylpolyamines are considered to be important intermediates in polyamine metabolism. In the vertebrate brain the putrescine to GABA pathway proceeds via acetylputrescine. There is also evidence for an active contribution of putrescine to GABA levels in developing nerve tissue (Seiler, N., Neurochem. Intl., 3:95, 1981). The acetylation of polyamines was examined in the following fractions of the developing and maturing mouse brain, whole homogenate, crude nuclei (P1), crude mitochondria (P2), microsomes and cytosol.

During the perinatal period (day 20 of gestation to day 4 after birth, a.b.), polyamine acetylation in whole homogenate was found to be high. Following a peak at day 5, the activity declined towards adult values.

In P1 and microsomal fractions acetylation of spermidine was high at day 1 and then declined rapidly to nearly adult values by day 10. Putrescine acetylation exhibited an additional peak at day 4.

In contrast to the adult animal (90 days), putrescine and spermidine acetylation was present in the P2 and soluble fractions. Acetylation of spermidine in the P2 fraction is highest at day 1, while putrescine acetylation is highest at day 3. Acetylation of both substrates is barely detectable at day 42 a.b.

Putrescine and spermidine acetylation in the soluble fraction showed the highest activity at day 5 a.b. after which there was a fast decline towards adult values.

These results suggest that polyamine acetylation may play an important role in polyamine metabolism in the developing mouse brain. The activity found in the P2 and soluble fractions strongly suggests that putrescine, via acetylputrescine, contributes significantly to the GABA levels during development.

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49.18 ENHANCEMENT OF RESPONDING FOR ELECTRICAL STIMULATION OF BRAIN BY COCAINE IN 10 DAY OLD RAT PUPS. Gordon A. Barr*, Leo Maniachi*, and Ted Lithgow* (SPON: W. H. Bridger). Dept. of Psychiatry, Albert Einstein College of Medicine and the Biopsychology Program, Hunter College, CUNY, New York, New York 10461.

In adult rats, stimulant drugs lower threshold and increase responding for brain stimulation reward (BSR). It has been proposed that these drugs may act through enhancement of catecholamine function. Recently, it has been shown that rat pups as young as 3 days of age will also work for BSR. Moreover, although many neurochemical systems, including the catecholamines, are not fully developed in preweanling rats, we have shown enhancement of responding for brain stimulation following amphetamine treatment in 10 day old rat pups. In the present study, we tested a second stimulant, cocaine, on BSR in this age pup.

Using stereotaxic methods developed for immature rats, 150 μ bipolar electrodes were aimed at the medial forebrain bundle. About 16-18 hours postoperatively, pups were placed in a small heated chamber (13 X 12 cm) with two omni-directional poles protruding from the floor. Nudging one pole produced a 280 msec pulse train (2.0 V, 125 Hz), while nudging the second pole had no consequence. Responses were recorded hourly. After the fifth hour, pups were injected i.p. with either cocaine (10 or 30 mg/kg) or saline and responses recorded for an additional five hours. Increased responding on both poles indicated increased activation; differentially enhanced responding on the active pole indicated enhancement of the stimulation.

The highest dose of cocaine enhanced responding differentially. The number of responses on the positive pole increased six-fold in the hour following injection while responding on the neutral pole only increased slightly. At the lower dose, responding on both poles increased from two to three-fold. Responding did not change on either pole following saline injection.

These results demonstrate that cocaine, like amphetamine, can enhance BSR in 10 day old rat pups. Cocaine differs from amphetamine only in having a shorter duration of action in these studies. For both drugs, higher doses were required in pups than typically used in adults to increase responding for stimulation. The neurochemical systems mediating the actions of the two stimulants on BSR are functional in this particular as early as 10 days of age.

49.19 NALOXONE INCREASES PREFERENCE FOR STRESS-RELATED ODORS IN RAT PUPS. C.A. Cornwell-Jones, Dept. of Psychology, Princeton Univ., Princeton, NJ 08544.

According to the affiliative need hypothesis, naloxone increases perceived stress by blocking opiate receptors, and developing animals seek out conspecifics in an effort to relieve the stress (cf. Panksepp et al., Neurosci. Biobehav. Rev., 4, 1978). This hypothesis suggests that naloxone should increase pups' preference for odors associated with the home-cage, but not other odors.

Experiments 1 and 2 examined the effects of naloxone injection on preference for home-cage vs. other odors. Sprague-Dawley rat pups 10-12 days old were injected with 0.1 ml of 1 μg/g naloxone HCl or saline and replaced in their home cages. Control pups were uninjected. In Expt. 1, home cages contained pine shavings, and in Expt. 2, cages contained cedar shavings. Seven min after injection, pups were tested in an apparatus with a screen floor which allowed pups to smell but not touch or taste shavings in 2 compartments below. One compartment contained fresh pine shavings. The other contained either fresh cedar shavings or soiled pine shavings from the nest of a strange litter. Each pup was tested on both odor choices.

Naloxone affected preference for home-cage, but not non-home-cage odor in both experiments. Pine-reared controls and naloxone treated pups preferred pine-nest to pine odor, while saline-injected pups were indifferent. Cedar-housed control and naloxone-treated pups preferred cedar to pine odor, while saline-injected pups showed no preference. Thus, naloxone did not increase preference for home-cage odors over control levels, but prevented the injection procedure from reducing that preference.

A final experiment examined naloxone effects on preferences for non-home odors encountered under stressful vs. non-stressful conditions. Following saline or naloxone injection, pine-reared pups were either placed in pine shavings under a heating pad, placed in lemon-scented shavings in a heated jar, or placed in lemon-scented shavings in a refrigerated jar. Animals were returned to their home cages after 30 min. and tested 24 hrs later on cedar vs. pine and lemon vs. pine.

All groups preferred pine to lemon-scented shavings except the group injected with naloxone and exposed to refrigerated lemon shavings 24 hrs earlier. This group showed no preference on the lemon vs. pine test, and averaged significantly more time over lemon odor than cold-exposed saline-injected pups or unexposed naloxone-injected pups.

The combined data suggest that naloxone increases pups' preference for odors encountered under stressful conditions. These results are consistent with the idea that naloxone acts as a false opiate in immature rats.

49.20 DEVELOPMENT OF BENZODIAZEPINE BINDING SITES IN DIFFERENT REGIONS OF THE FETAL RAT BRAIN. M. Schlumpf, J.G. Richards, W. Lichtensteiger and H. Moehler*. Inst. of Pharmacology, Univ. of Zurich, Zurich, and F. Hoffmann-La Roche, Basle, Switzerland.

Benzodiazepines (BDZ) are known to readily cross the placental barrier when administered to pregnant rats. Administration of BDZ during pregnancy results in subtle changes in the behavior of offspring. The availability of novel BDZ ligands with different pharmacological profiles, in particular the specific BDZ antagonist Ro 15-1788, provides an opportunity to investigate the ontogeny of pharmacologically relevant BDZ binding sites (receptors) in fetal rat brains.

Zivic Miller-Sprague Dawley rats were mated for 1½ hr at the onset of the dark period (embryonic day (ED) 1 is 24 hr after mating). An in vitro autoradiographic method (Young and Kuhar, Brain Res. 179, 255 (1979)) was used to visualize the binding sites of ^{3}H-flunitrazepam and ^{3}H-Ro 15-1788 in cryostat sections from fetal brain. The specificity of radioligand binding was checked by co-incubating sections with an excess of the non-radioactive antagonist or clonazepam respectively.

Specific BDZ agonist and antagonist binding was detected on ED 14 in the spinal cord and lower brain stem. Between ED 14 and 15 labelling spread throughout the lower brainstem, the mesencephalon and parts of the diencephalon. On ED 16 binding was present in the developing caudate putamen, olfactory bulb and the frontolateral parts of the neocortex. In neocortex only the most superficial cortical layer appeared labelled at this stage of development. During the later stages of prenatal development the density of BDZ binding in the di- and telencephalon increased and developed into an adult-like pattern. In the neocortex, layer I appeared as a distinctly labelled band, while very little or no labelling was seen below the cortical plate. In general, a caudo-rostral gradient was observed in the development of BDZ binding sites in fetal rat brain.

The capacity of the fetal brain to specifically bind BDZ may be related to the functional deficiencies described in the offspring of BDZ-treated mothers. Our observations suggest that different brain structures may become sensitive to the action of BDZ at different times in prenatal development.

50.1 ALTERED CENTRAL AND SOMATIC DEVELOPMENT IN MALNOURISHED ARTIFICIALLY REARED RAT PUPS. J. Diaz, E. Moore*, C. Stamper, F. Petracca*. Dept. of Psychology, University of Washington, Seattle, WA 98195.

The procedure of rearing rat pups away from their mother and siblings exclusively by intragastric feedings provides a means for direct examination of nutritional requirements for normal development. This technique allows separation of malnutrition from undernutrition which is not possible using litter size or maternal diet manipulations. Rat pups artificially reared days 4 through 18 have been found to differ significantly from their normally reared siblings on day 18 in specific organ weights despite similar whole body growth. The purpose of this study was to examine the development of these differences.

Four-day old female Long Evans hooded rat pups were assigned by weight to either a normally reared group (NR) or an artificially reared group (AR). Both groups were further subdivided according to the age at which they were to be sacrificed : day 5,6,8,12, or 18. Animals assigned to the AR group were reared using a formula accepted as a substitute for mother's milk, according to a procedure previously described (Diaz, et. al, J. Nutr., 1982). On the day of sacrifice, each animal's brain, liver, kidney, and spleen were removed and weighed. The brain was dissected and the cerebellum weighed.

The following table summarizes the organ weight data for the AR group compared to the NR group :

	DAY 5	DAY 6	DAY 8	DAY 12	DAY 18
WHOLE BRAIN	-9%	-8% *	-11% **	-11% **	-8% **
CEREBELLUM	-3%	-8%	-9% *	-17% **	-7% **
LIVER	+6%	+13% **	+21% **	+21% **	+34% **
KIDNEY	-7%	-6%	+10%	+12% *	+27% **
SPLEEN	-18%	-32% **	-25% **	+17%	+37% **

(* P<.05; ** P<.01)

The malnutrition in the AR group was so severe that significant deficits in brain weight could be observed after 48 hours of gastric infusions. The subsequent brain growth of the AR animals showed its largest deficit during the time of fastest brain growth. The decreased deficits seen on day 18 in the AR group suggest that these animals may experience a recovery period of enhanced brain growth. The specific nature of the malnutrition responsible for such a brain weight deficit remains unclear. However, the accelerated liver growth in these animals suggests the presence of a toxin or an inappropriate component in the formula.

Recent experiments in our laboratory examining different formulas suggest that the pattern of reduced brain growth and accelerated liver growth reported in the present study is not a necessary consequence of artificial rearing.

50.2 THE EFFECTS OF PRENATAL AND POSTNATAL MALNUTRITION ON BRAIN LIPIDS. Paula K. Lundberg, C. Robert Almli, Richard W. Florman* and Peter J. Morgane. Washington Univ. Sch. of Med., St. Louis, MO 63110 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Pre- and postnatal (PN) malnutrition has been shown to be associated with a variety of deficits in rats. Typically, the brains of rats that have been malnourished postnatally show reductions in the total amount of cholesterol (CHOL) and phospholipids (PHOS) as well as reductions in the concentrations of these lipids. With the exception of the "large litter" technique, these results occur regardless of the method used to effect malnourishment. Since we have been interested in developing an animal model of the small-for-gestation-age infant utilizing a 6% protein diet, determination of the diet's effects on brain lipid levels is important for the explanation of its behavioral consequences. The total lipid content in the brain can be considered an index of general growth, while the concentrations of brain CHOL and brain cerebroside and sulfatide can be considered an index of myelination. Thus, the purpose of the present study was to determine the effects of pre-and PN exposure to a 6% protein diet on offspring lipid levels.

Prior to breeding, during gestation and lactation dams were maintained on normal (N) (25% casein) or low-protein (LP) (6% casein) isocaloric (4.3 kcal/g) diets. After weaning, offspring were maintained on their respective diets. Pups were sacrificed on PN days 21 or 35, their brains were removed, dissected into four parts and frozen for lipid analysis. CHOL was analyzed using a Biodynamics Kit. PHOS was analyzed using the method of Fiske and Subbarow. Analyses of cerebroside and sulfatide as well as analysis of the other brain regions are currently in progress.

The weights of the telencephalon were reduced in both the day 21 (t=13.58, df=22, p .001) and day 35 (t=10.03, df= 30, p .001) LP rats. Telencephalon CHOL concentrations (mg/g brain) in the day 21 LP rats were not different from those of the N rats, but total CHOL content (mg) was lower in the LP rats (t= 4.80, df=21, p .001). In the day 35, LP rats, however, both the concentrations of CHOL (t=3.63, df=29, p .01) and total brain CHOL (t=7.33, df=29, p .001) were less than those of the normals. Total PHOS levels (mg) in the telencephalon were reduced in the day 21 LP rats only (t= 2.34, df=16, p .05).

These data suggest that exposure to a 6% protein diet results in impaired brain growth since brain weight, brain CHOL and PHOS levels are lower at weaning. Moreover, exposure until day 35 results not only in reductions in brain weight and total CHOL, but also CHOL concentration implying that myelination is also reduced. (Supported by BSRG grant SO7RR05389, NICHD grant HDO6364 and NHLBI grant HL07456-01.)

50.3 ALTERATIONS IN NEURONAL DISCHARGE RATES IN DEVELOPMENTALLY PROTEIN MALNOURISHED RATS. W. C. Stern, A. Johnson*, O. Resnick and P. J. Morgane. Neuropharmacology Lab., D. Dix Hosp., Raleigh, N.C. and Worcester Fndn. Exp. Biol., Worcester, MA.

There has been little electrophysiological evaluation at the single unit level of changes in the CNS in rats reared under conditions of protein malnutrition. The present study examined in normal adult rats fed a 25% casein diet and developmentally protein malnourished rats fed an 8% casein diet: (a) the spontaneous discharge rates (and inter spike intervals) of neurons in the parietal cortex and medial thalamus and (b) the effects of electrical stimulation of the locus coeruleus (LC) and raphe dorsalis (RD) inputs to these neurons. The isocaloric diet regimens were initiated prior to conception and continued throughout the gestation, lactation and post-weaning periods until testing in adulthood. Neuronal activity was recorded extracellularly in urethane anesthetized rats using glass micropipette electrodes. Following recording of baseline activity, stimulation (10 Hz, 0.1-1.0 mA, trains of biphasic pulses) of 10-30 sec was applied to the LC or DR.

The results were: (a) The spontaneous discharge rates of populations of cortical and thalamic units were substantially different in protein malnourished rats compared to the normal controls. The protein malnourished group had a virtual absence (5-7% of all units) of fast-firing cells (>20 spikes/sec) compared to the normal controls (31-42% of units). Consequently, there was a 30-50% decrease in average discharge rates in the malnourished group. (b) Activation of the aminergic pathways by electrical stimulation of the LC or DR produced comparable patterns of changes in neuronal firing rates of the cortical and thalamic units in normal and protein malnourished rats.

The most important aspect of the present findings was the very substantial reductions in the proportion of forebrain units with a fast spontaneous firing rate (>20/sec) in the protein malnourished rats. The basis of this effect is not known, but could be related to the enhanced levels of norepinephrine and serotonin previously reported to occur in these animals. Alternatively, the malnourished rats may have a deficiency in an excitatory neurotransmitter. The extent of the present changes in spontaneous discharge rates in other brain regions and the underlying physiological abnormality responsible for these differences remains to be determined.

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50.4 MALNUTRITION AND THE DEVELOPMENT OF THE BRAIN: A TIMETABLE FOR REHABILITATION. A. G. Angulo-Colmenares, L. Briceño-Mayz* and J. L. Colmenares. Servicios de Anatomía Patológica y Endocrinología, Hospital Militar "Dr. Carlos Arvelo", Caracas, Venezuela.

Previous work has shown that, when proper nutrition is provided, rats malnourished during lactation reach normal body weight and attain a normal size to their cerebral hemispheres by 70 days of age and have normal values of cerebral cortex thickness by 40 days (Angulo-Colmenares, A. G., J. W. Hinds and D. W. Vaughan, Brain Res. 169:121-138, 1979). We now report on our attempt to rehabilitate rats malnourished perinatally until 30 and 40 days of age.

Pregnant Sprague-Dawley rats were given either a 24% (control) or 8% (experimental) protein diet starting at day 10 of gestation and continuing until 30 (R30) or 40 (R40) days after birth. At this time rehabilitation was induced by feeding the animals a 24% diet. In addition, the R30 group, which was still lactating at 30 days, was left with their pups until day 40 and the litter was reduced from 8 to 4 pups. Throughout the experiment a group of animals was kept malnourished by feeding them the 8% diet. Observations were made on tissue from animals 40, 70 and 90 days old fixed by vascular perfusion of aldehydes and embedded in Araldite.

Our results indicate that while a substantial recovery is made in terms of body weight, neither group achieves normal values by 90 days of age. Three dimensions of the cerebral hemispheres were measured: length, width and height. At 40 days all three dimensions are significantly reduced in the malnourished animals. These differences have disappeared in the R30 group at 70 days of age. In the R40 group the length and height are no longer different at 70 days but the width is still significantly reduced in the 90 day animals. Preliminary results suggest recovery of cerebral cortex thickness in the R30 animals by 70 days while the R40 group apparently did not recover by 90 days.

We conclude that in this particular model of perinatal malnutrition, the manipulations that induce rehabilitation in the brain are no longer effective after 40 days of age. The question remains if the stimulation of growth by pharmacological manipulations and/or hyperalimentation may further increase the possibilities of rehabilitation. (Supported by Consejo Nacional de Investigaciones Científicas y Tecnológicas, proyecto CICS-4)

50.5 THE EFFECTS OF PRENATAL PROTEIN DEFICIENCY ON THE DEVELOPMENT OF THE CORPUS CALLOSUM IN THE BALB/C LABORATORY MOUSE. P. Wainwright* R. Stefanescu* (SPON: R. Marteniuk). Dept. of Health Studies, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

The study compared the effects of an 8% vs 27% casein diet during gestation on forebrain fibre tract development in the laboratory mouse. The Balb/c strain was used because it is prone to having a defective or absent corpus callosum. Females were assigned to the experimental diets either two weeks prior to mating (chronic) or on the seventh day of gestation (acute). Fetal brain development was assessed at 18.5 days after conception. The data indicated that protein deficiency increased the number of animals who did not have a corpus callosum present in a midsagital section (chronic 14.7%, acute 23.9%, control 7.4%; $\chi^2_{(2)} = 5.35$, $p = .05$). In addition, in those animals in whom the structure was present, it was evident that the cross sectional area was smaller in the chronically deprived animals than in the controls. Covariance analysis indicated that this effect was independent of effects on other variables such as brain weight and area of the anterior commissure. This suggested that the smaller cross sectional area was not merely evidence of growth retardation but was an effect specific to the corpus callosum.

50.6 EFFECT OF PRENATAL PROTEIN MALNUTRITION UPON THE CORTICAL AND HIPPOCAMPAL EEG IN THE DEVELOPING RAT. J.D. Bronzino, M. Kelly,* K. Austin,* C. Cordova,* C. Siok,* O. Resnick and P.J. Morgane Worcester Foundation For Experimental Biology, Shrewsbury, MA 01545.

Although many studies have shown that dietary protein deficiencies during the period of most rapid brain growth causes significant and often irreversible alterations in the structure, chemical composition and function of the brain, the effects of protein malnutrition on ontogenesis of electrical activity in specific neuronal formations is not well understood. Since developmental processes undergo definite ontogenetic sequences, electrographic measures in normal animals can serve as baseline indicators against which those obtained during various insults to the brain can be compared. Recently, we have demonstrated (Brain Res. Bull. 5:51-60, 1980) the effects of prenatal protein malnutrition upon the frequency characteristics of the EEG as the developing rat matures from early post-weaning to adulthood. In the present studies we undertook a quantitative study of the developing EEG in normal (25% casein diet) and protein malnourished (8% casein diet) rats during the preweaning stage of development. Specifically we utilized power spectral techniques to quantify EEG patterns in several vigilance states as the animal matured from 14 to 22 days of age. Although changes in the frequency characteristics, especially at lower frequencies (0.5 - 3 Hz), were observed in the cortical EEG, the most significant findings of this study involved the theta rhythm (4 - 11 Hz) in the hippocampal EEG. In normal development, the power in the 4 - 7 Hz band gradually increases while power in the 7.5 - 11 Hz band gradually decreases and the frequency at which the peak power value occurs continually shifts to higher values (i.e., from 5 to 6.5 Hz) in this preweaning period. The protein malnourished animals had significantly less power in the theta band of frequencies at 14, 18 and 22 days of age. In addition, the gradual shift in the frequency of the peak power for the protein malnourished animals lagged behind those of the animals reared on the 25% casein diet until 22 days of age when the frequencies were observed to be approximately equal between the two diet groups. These results indicate that the electrographic activity recorded from the hippocampus in animals reared on a protein deficient diet are electrographically "immature" during preweaning, i.e., at 14, 18 and 22 days of age, and demonstrate that prenatal protein malnutrition can significantly alter specific electrographic measures related to theta activity. (Supported by NIH Grant NICHHD HD06364.)

50.7 ALTERATION OF REM EPISODES IN THE DEVELOPING RAT PRODUCED BY PRENATAL PROTEIN MALNUTRITION K. Austin,* J.D. Bronzino, M. Kelly,* C. Cordova,* C. Siok,* O. Resnick and P.J. Morgane (SPON: R. Volle) Worcester Foundation For Experimental Biology, Shrewsbury, Massachusetts 01545.

In order to for understand the functional implications of organic effects of protein malnutrition on the developing brain, we have studied sleep behavior in normal and malnourished rats at various times of life. We have previously reported the effects of prenatal protein malnutrition upon the sleep profiles of adult rats (Expl. Neurol. 57:440-450, 1977). In the present study we examined the sleep profile in rats reared on a normal (25% casein) diet and a protein deficient (8% casein) diet during the preweaning stage of development, particularly 14 to 22 days of age. Chronic indwelling bipolar recording electrodes were implanted in frontal cortex and hippocampus under ether anesthesia at 14, 18 and 22 days of age. Following surgery the animals were placed into a sleep chamber, and after recovery from anesthesia they were recorded for 6 hours to establish the sleep profile for each animal. The most significant finding of this study involved REM sleep. At 14 and 18 days of age, protein malnourished rats spent significantly less time in REM sleep, but by 22 days of age had approximately the same REM sleep percentage displayed by those animals reared on the 25% casein diet. Although less time was spent in REM sleep in animals 14 and 18 days of age, these protein malnourished animals had a significantly higher number of REM episodes when compared to normals, i.e., the animals exposed to prenatal protein malnutrition experienced more frequent REM bouts but spent much less time in REM sleep than those animals reared on a normal diet. These results demonstrate effects of dietary prenatal protein restriction on the REM sleep state and the overall sleep-waking process. The effects are restricted to the REM sleep state and do not involve slow-wave sleep. Since prenatal protein malnutrition alters the sleep-waking characteristics of the rat during preweaning, i.e., at 14 and 18 days of age, derangements in those neuronal structures involved in the REM sleep control system, may be caused by prenatal protein malnutrition. (Supported by NIH Grant NICHHD HD06364)

50.8 THE EFFECT OF PROTEIN MALNUTRITION ON THE COPULATORY BEHAVIOR OF THE MALE RAT. R.D. Hall, B.J. Feldman* and J. Flemming*. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

In this laboratory Forbes and Svare (in preparation) found that developmental protein malnutrition led to abnormally high levels of plasma testosterone (T) in rats 240 days of age. At that age and at 120 days testes and seminal vesicles were also relatively heavier in protein deprived rats than they were in well-nourished ones, but plasma T levels were not elevated in the younger animals. The purpose of this study was to determine whether the morphological and hormonal differences between the protein malnourished and well-nourished rats had behavioral correlates and, if so, whether they were age related.

In three experiments rats were reared under four dietary conditions: Half of the rats were born to dams fed a low protein (8% casein) diet from 5 weeks before mating until their pups were weaned at 21 days of age. The other half was reared by dams fed a 25% casein diet. At weaning half of the pups in each group were switched to the other diet.

In the first experiment 32 males were given a single test of copulatory behavior at each of two ages, 165 and 325 days. In the second experiment 46 males were given as many as four tests to achieve ejaculation at 170, 215 and 305 days of age. In the final experiment 40 males were allowed three tests beginning at 95 days of age.

In all three experiments rats subjected to the protein deprivation in both preweaning and postweaning periods, or in one or the other only, were more effective copulators than well-nourished rats. This was evident in several measures of intromission responses: the number of intromissions, interintromission intervals, and the proportion of copulatory attempts that achieved intromission. The greater success of the protein malnourished rats in achieving intromission also resulted in more ejaculations at the older test ages. The diminished capacity for intromitting associated with the high protein diet also occurred with increasing age. In neither case did other measures of the copulatory behavior suggest that sexual arousal was decreased. These data suggest that penile reflexes may be enhanced by elevated T levels in protein malnourished rats, although other factors may also be involved. The more vigorous copulatory behavior of malnourished 95-day-old males, which presumably have normal plasma T levels, suggests that other factors are important.

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50.9 DIETARY OBESITY IN WEANLING RATS WITH AND WITHOUT DORSOMEDIAL HYPOTHALAMIC LESIONS (DMNL). Lee L. Bernardis and Larry L. Bellinger. VA Med. Ctr. Buffalo and SUNY at Buffalo, NY 14215 and Dept. Physiol. Baylor Coll. of Dent., Dallas TX 75246.

Recent studies by others have shown that, although post-weaning intact rats fed cafeteria diets eat 50% more calories (CAL) than chow-fed rats they gain only slightly more weight (BW). Their feeding efficiency (EFU) is reduced and their brown adipose tissue (BAT) weight is increased; their capacity for dietary-induced thermogenesis (DIT) appears as great as that of mature rats. Weanling male rats received DMNL and sham operations (CON) and were fed lab chow for 15 days post-operatively (POP). During this time they showed the previously reported hypophagia and BW and length retardation. Following this, they were fed a high-fat diet, sucrose as drinking fluid, cookies and assorted "junk food" for various periods until the 185th POP day. In most parameters, our findings are in contrast to those in slightly older (5 d) rats. Thus, CON ate the same CAL or less than chow-fed CON. DMNL rats and their chow-fed counterparts ate comparable CAL. EFU was greater in both CON and DMNL rats fed "junk food" compared with CON and DMNL fed chow. However, CON and DMNL rats under each dietary treatment had comparable EFUs. BW as such was consistently reduced in DMNL compared to CON. Except during the "junk food" period, CON weighed less than chow-fed CON. However, DMNL rats fed the special diets in whatever combination weighed consistently less than chow-fed DMNL rats. Change in BW, i.e. ΔBW, was greatly increased in the two "junk"-fed groups; this was paralleled by increased plasma levels of glucose, glycerol and free fatty acids at sacrifice. BAT weight was increased in the CON fed the special diets compared to chow-fed CON, but was similar in the two DMNL groups. BAT weight of DMNL on the special diets was also reduced compared to special diet-fed CON but there was no difference in BAT weight between chow-fed DMNL and CON. BAT lipid content was comparable in the special diet-fed groups and in turn was greater in these two groups than in their chow-fed counterparts. Epididymal fat pads weighed more in the special diet-fed groups than in the chow-fed groups but, as with BAT, the special diet-fed DMNL rats showed lower epididymal pad weights than their CON. The epididymal pads in the chow-fed groups were comparable in DMNL and CON. Linear growth was retarded in both DMNL groups compared with their CON but, most remarkably, was dramatically reduced in both special diet-fed groups compared with their chow-fed counterparts. Manifestations of dietary obesity and DIT differ in weanling rats from slightly older and mature rats. DMNL bring about additional differential responses that point to yet to be identified deficits in DIT.

Supported by VA Funds.

50.10 PRENATAL PROTEIN MALNUTRITION IN RATS: INFLUENCES ON BRAIN NOREPINEPHRINE SYNTHESIS. Oscar Resnick, Rachelle Hasson* and Maravene Miller. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We have reported significant elevations in regional brain norepinephrine (NE) levels from birth through adulthood in rats whose dams ingested a low protein diet (8% casein) starting 5 weeks prior to conception as compared to progeny from dams fed a normal diet (25% casein). These alterations appeared to be correlated with increases in phenylalanine (Phe) availability to the brain rather than with elevations in brain or plasma tyrosine (Tyr) levels (Miller et al., Exp. Neurol., in press, 1982). To determine which of the brain and peripheral changes were due to prenatal protein deficits and which were caused by lactational deficiencies, rats born to dams fed the 8% or 25% diets were cross-fostered at birth to dams of the opposite diet, i.e., pups from 8% dams were fostered on 25% dams (8/25 rats); 25% pups were fostered on 8% dams (25/8 rats). At weaning (day 21), these rats were compared to unswitched pups of each diet (8/8 or 25/25 rats). The results (table below) show that while some constituents can be induced as a sequela of lactational deficits, many were not rehabilitated by a sufficient diet during lactation. Accordingly, adequate nutrition instituted at birth was ineffectual in reversing the prenatal-determined alterations in brain NE synthesis. While the mechanism(s) responsible for these changes are unknown, these data demonstrate that prenatal protein malnutrition alone can importantly influence brain catecholamine metabolism in the postnatal period.

Influences of Pre- and Postnatal Diets at 21 Days of Age

Pre/Post	8/8	25/8	8/25	25/25
Brain				
NE ng/g	360 ± 10	318 ± 8	349 ± 9	208 ± 5
Tyr ng/g	18711 ± 247	19161 ± 293	23388 ± 327	24079 ± 572
Phe ng/g	24059 ± 251	23958 ± 334	23852 ± 499	15735 ± 271
Weight mg	1578 ± 58	1530 ± 92	1776 ± 33	1815 ± 40
Plasma				
Try ng/ml	18718 ± 541	18186 ± 273	28176 ± 454	27274 ± 749
Phe ng/g	11361 ± 345	9371 ± 138	11961 ± 392	7978 ± 91
Alb* μg/ml	2842 ± 38	2911 ± 33	3721 ± 49	4692 ± 102
Pro† μg/ml	4124 ± 78	4103 ± 28	6037 ± 28	6052 ± 51
Body Wgt. g	25.8 ± 1.6	27.7 ± 0.5	63.5 ± 1.0	61.8 ± 1.9

*Alb = Albumin; †Pro = Total Protein

Supported by grant HD 06364

50.11 TRYPTOPHAN AVAILABILITY: ALTERATIONS IN BRAIN INDOLEAMINE METABOLISM IN RATS AS SEQUELAE OF INTERGENERATIONAL PROTEIN MALNUTRITION. Maravene Miller, Rachelle Hasson* and Oscar Resnick. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The second filial (F_2) generation of protein deprived rats, whose dams were exposed to an 8% casein diet from their own conception (8-F_1 rats), showed a small-for-gestational-age syndrome at birth (>30% decreases in body weight) as a response to their dams' poor nutritional status. This severe in utero malnutrition caused the 8-F_2 pups to show significant elevations in brain serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and tryptophan (TRP) at birth (Table below) compared to 25-F_2 rats whose dams were exposed to an adequate diet (25% casein) from their conception. After birth, the poor milk production of the 8-F_1 dams caused their pups to display the failure-to-thrive symptoms (>60% decreases in body weight) of infantile marasmus by day 11 of lactation. Also, these lactational deficits were responsible for the 16-190% increases in brain indoleamine metabolism of the 8-F_2 pups versus the 25-F_2 rats during lactation (days 5-21). Interestingly, both groups of F_2 pups had significantly higher values for their brain 5-HT, 5-HIAA, and TRP values at birth and other ages compared to F_1 rats (Exp. Neurol. 57: 142, 1977). These increases were 29-495% for the 8-F_2 vs 8-F_1 pups and 9-142% for the 25-F_2 vs 25-F_1 rats. These data indicate that the availability of TRP to the developing brains of F_2 offspring can be increased under conditions of either chronic lack or super abundance of protein in the F_1 maternal diet.

	Day of Birth (Mean ± SE)	
Diet Group	8-F_2	25-F_2
(n)	(8)	(8)
	5-HT ng/g	
Telencephalon	757 ± 43[a]	444 ± 39
Brainstem	913 ± 31[a]	664 ± 15
	5-HIAA ng/g	
Telencephalon	1121 ± 75[a]	716 ± 56
Brainstem	1196 ± 57[a]	843 ± 6
	TRP ng/g	
Telencephalon	19645 ± 1091[a]	12514 ± 993
Brainstem	17764 ± 591[a]	12170 ± 850

[a] $p < 0.001$, 2-tailed t-tests (Supported by grant HD 06364)

50.12 GANGLIOSIDES IN BRAIN REGIONS FROM THE DEVELOPING OFFSPRING OF CONTROL AND ETHANOL PUPS. M.J. Druse-Manteuffel, A.B. Noronha* B.G. Oden* and R.G. Haas* (SPON. J.H. Hofteig). Department of Biochemistry, Loyola University Medical School, Maywood, IL 60153.

The present study examined gangliosides (GA) in 6 brain regions - cerebral cortex (CX), cerebellum (CB), hippocampus (HIP), hypothalamus (HT), brain stem (BS) and corpus striatum (CS) - from the 10 to 24 d. offspring of control (C) and ethanol (E)-treated female rats. Female rats were pair-fed, using C or 6.6% (v/v) E liquid diets which contained 21% protein (Sub. Alc. Abuse/Misuse 2: 359, 1981), either on a chronic basis (prior to and during gestation) or on a short-term basis (3rd trimester of gestation). Offspring of 'chronic' mothers were given an intracerebral injection of [^{3}H]- or [^{14}C]-N-acetylmannosamine 16 h prior to sacrifice . GA were extracted (Life Sci. 3: 1227, 1964) and separated by thin layer chromatography (TLC) (Lipids 15: 1055, 1980). GA were quantitated by scanning resorcinol-treated TLC plates (BBA 24: 604, 1975). The relative distribution of radioactivity among brain region GA was determined by liquid scintillation counting.

We noted developmental changes in C rats in the proportion of several GA. 1) GQ_{1b} decreased in the BS & HIP. 2) GT_{1b} decreased and GM_1 increased in the CX. 3) GD_{1b} increased in the BS. Although GD_{1a} & GT_{1b} were major GA in each brain region examined, $GD_{1a}/GT_{1b} > 1$ in the CX, HIP & CS, while the ratio was ~ 1 in the HT, BS & CB.

In comparison to C pups, the offspring of chronic 'alcoholic' rats demonstrated several significant differences in the relative distribution of GA and associated radioactivity in the different brain regions. The most notable differences were the increased proportion of GT_{1b} in the CS & HIP and the decreased proportion of this GA in the CX of 10 d E pups. In addition, developing E pups demonstrated a consistent decrease in the proportion of GD_{1b} in the CS and a transient decrease of GD_{1a} in the HT. The relative synthesis of GD_{1b} was decreased in the HIP, CS and CB in 10 d E pups, whereas that of GT_{1b} was transiently increased in the HT, BS, CS & CB. Although all brain regions demonstrated GA abnormalities in the E pups, the greatest differences were found in the HIP & CS.

In contrast to the offspring of chronic 'alcoholic' rats, there were no differences in the distribution of brain region GA in the offspring of rats that were treated with E on a short-term basis. However, the latter group of animals demonstrated an increased concentration of total GA in the BS & HIP at 10 d.

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50.13 GANGLIOSIDES IN AXOLEMMA FROM DEVELOPING CONTROL AND ETHANOL PUPS. J.M. Gnaedinger* and M.J. Druse-Manteuffel. Department of Biochemistry, Loyola Univ. School of Medicine, Maywood,IL 60153.

Female rats were pair-fed using either control(C) or 6.6% (v/v) ethanol(E) liquid diets, containing 21% protein (Sub. Alc.Abuse/Misuse 2:359,1981), on a chronic basis prior to conception and during gestation. Rats were injected intracerebrally with either [^{14}C]- or [^{3}H]-N-acetylmannosamine 16 hours prior to sacrifice. Axolemma fractions were isolated (J. Neurochem. 34:424,1980) from the brains of 20- to 34-day-old offspring of control and ethanol pups. Gangliosides were extracted (Life Sci.3:1227,1964) from axolemma and separated by thin layer chromatography (Lipids 15: 1055,1980). The relative distribution of radioactivity among axolemmal gangliosides was determined by liquid scintillation counting.

Although we did not observe statistical differences in the relative distribution of radioactivity among axolemmal gangliosides from C & E pups, we did observe developmental changes in the ganglioside patterns. At all ages, the largest proportion of radioactivity was found associated with gangliosides GD_{1a} & GT_{1b}. However, whereas GD_{1a} was most heavily labeled at 20 days (∿30%), GT_{1b} had the major proportion of radioactivity at 34 days (∿33%). (The percentage of radioactivity associated with GD_{1a} at 34 days was ∿21% and that of GT_{1b} at 20 days was ∿20%.) Several other developmentally-related trends were observed in the axolemma from both C & E offspring: 1) GQ_{1b} increased from ∿5% at 20 days to ∿10% at 27 and 34 days; 2) GM_1 decreased from ∿10% at 20 days to ∿5% at older ages. The low concentration of monosialogangliosides and the high ratio of GT_{1b}/GD_{1a} in axolemma from mature animals are distinct from the ganglioside patterns of CNS myelin and synaptic membranes , respectively.

An additional interesting observation was the increased yield of axolemma from 20-day-old E pups. Although the axolemma isolation procedure is not quantitative, this observation could be important in light of our previous report of increased CNS myelin (particularly the heavy subfraction) in young E pups (Drug Alc. Depend. 2: 421,1977).

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50.14 MYELIN RECOVERY FROM FETAL AND PERINATAL ETHANOL EXPOSURE. F. E. Lancaster, S. Phillips* and R. C. Wiggins. Department of Biology, Texas Woman's University, Houston, Texas 77030 and Department of Anatomy and Neurobiology, University of Texas Medical School, Houston, Texas 77030.

Timed pregnant Long Evans rats received four dietary regimes from the fifth of gestation throughout lactation: ethanol (5.15%) liquid diet, pair fed liquid diet control, ad lib liquid control, and ad lib lab chow. Pups were cross fostered at birth such that each litter contained eight pups with two original pups and two pups from each other dietary group. Pups were sacrificed at 16, 21, 30 and 52 days of age. Whole brain myelin accumulation was determined quantitatively using preparative ultracentrifugation. Eye opening was delayed and birth weights were lower in the ethanol pups. Myelin accumulation, brain weights, and body weights were depressed in all pups cross fostered to ethanol treated dams during lactation. Pups born to ethanol treated dams also lagged slightly behind other pups when they were cross fostered to pair fed and normal dams. Recovery (after weaning) in body weights and myelin accumulation remained incomplete at 52 days of age.

50.15 REORGANIZATION OF ACETYLCHOLINESTERASE HISTOCHEMICAL STAINING FOLLOWING AN ENTORHINAL LESION IS ALTERED IN ADULT RATS EXPOSED TO ETHANOL IN UTERO. S.L. Dewey and J.R. West, Dept. of Anatomy, College of Medicine, University of Iowa, Iowa City, IA 52242.

Mental retardation is the most deleterious feature of the fetal alcohol syndrome (FAS). Although delays in development may account for some of the reported mental deficiency, it is now clear that prenatal ethanol exposure can result in permanently altered neuronal circuitry (West et al., Science, 211:857, 1981). Since it is important to determine if there are other long-lasting effects of heavy maternal ethanol consumption, we chose to investigate whether the stereotypic reorganization in the dentate gyrus following entorhinal lesions (axon sprouting) was also altered in FAS animals. Within a week following an entorhinal lesion in an adult rat there is an intensification of acetylcholinesterase (AChE) staining in the outer molecular layer of the dentate gyrus and a concomitant increase in the width (and decrease in staining) in the commissural/associational (C/A) zone. We examined this response to similar lesions on the offspring from dams exposed to a liquid diet (Bio-Serv, PR-11) containing 35% ethanol derived calories during days 1-21 of gestation (a developmental period roughly equivalent to only the first and second human trimesters). Litters were culled to eight pups each at birth and cross fostered to normal mothers. They were weaned at 22 days of age and allowed to survive to an age of at least 60 days before receiving an electrolytic unilateral entorhinal lesion. Following at least 30 days survival, their brains were processed for AChE histochemistry using the Geneser-Jensen and Blackstad's modification of the Koelle procedure. The expansion of the C/A zone was significantly greater in the rats exposed to ethanol in utero compared with either normal or pair-fed controls with similar lesions as adults ($p<0.05$). The outer molecular layer in the FAS rats was intensely stained but was narrower than those of rats in either of the lesioned control groups ($p<0.01$). The C/A zone on the non-lesioned side of the ethanol-exposed rats was not significantly different from controls, suggesting that the observed post-lesion differences were probably not due to developmental differences in afferent lamination. Rather, these results suggest that prenatal ethanol may alter the ability of the cholinergic fibers to sprout or their competitive interaction with other afferent systems. Supported by grants AA03884 and AA05523 from NIAAA to J.R.W.

50.16 Prenatal Ethanol Exposure and Rat Hippocampal Development. D.E. Barnes and D. Goldberg.* Neurohistology Laboratory, Veterans Administration Medical Center and Univ. of Fla. Sch. Med., Gainesville, FL, 32610.

Previous studies indicated that prenatal ethanol exposure during the period of rat neurogenesis resulted in altered hippocampal and cerebellar development. Although litter size, birth weight and postnatal weight gain were normal for ethanol-treated animals, the number of hippocampal pyramidal cells were significantly reduced (Dev. Br. Res. 1:33). Thymidine autoradiography was employed to further examine the results of prenatal ethanol exposure.

Pregnant Long-Evans rats were maintained on an ethanol containing liquid diet (35% ethanol-derived calories) during days 10-21 of the gestational period. Control groups were given lab chow and water ad libitum or pair fed the liquid diet with sucrose substituted isocalorically for ethanol. Two or more animals from each group were injected with tritiated thymidine (New England Nuclear, specific activity 20 Ci/mM, 5 mCi p.g.w) on one day of gestation (Days 10-22). At parturition, the offspring were culled to 8 males and fostered to additional control mothers. Offspring were sacrificed at sixty days of age, the brains removed, coded to prevent experimenter bias and embedded in paraffin. Sections were taken at four microns, dipped in Kodak NTB-2, exposed for sixty days and developed in Dektol. Cell counts were made in the dorsal hippocampus of matched sections. The total and labeled cells of regions CA1 and CA3 were enumerated as well as the area of the dentate gyrus and total granule cells.

The lab-chow and sucrose-fed animals were identical in their pattern of labeling. The labeling index (percentage of labeled neurons) of regions CA1 and CA3 was high on gestational day 14, declining gradually to day 19 and dropping sharply on day 20. The ethanol-treated offspring had an erratic pattern of labeling compared to controls and had a significantly ($P<.01$) greater labeling index on gestational days 18 and 19. These results indicate that prenatal ethanol exposure may alter the developmental sequence in addition to the reduction in the numbers of hippocampal neurons.

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50.17 TAURINE DEPLETION WITH IMPAIRED CONE FUNCTION IN RHESUS MONKEYS. M. Neuringer*, J.A. Sturman, L. Feeney-Burns*, M.L. Klein*, and D.D. Denney* (SPON: L. Gronke). Oregon Regional Primate Center, Beaverton 97006, Oregon Health Sciences Univ., Portland 97201, and Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Taurine is an essential nutrient for the cat. Its absence from the diet produces plasma and tissue taurine depletion, reduced electroretinograms (ERG's), and eventual photoreceptor degeneration. Human newborns are partially dependent on dietary sources of taurine: plasma taurine levels are reduced by half when taurine-rich breast milk is replaced by artificial infant formulas containing ≤ 1 μmol% taurine. It is not known whether human vulnerability to taurine depletion persists beyond the neonatal period, or whether taurine depletion leads to functional changes in the retina or nervous system.

We fed an artificial human infant formula containing ≤ 1 μmol% taurine to five rhesus monkeys from birth. Four rhesus infants received the same formula supplemented with 70 μmol% taurine, the level in rhesus milk, and another four received rhesus milk and a stock diet. Until one year of age, plasma taurine levels of the taurine-deprived group were reduced by half. At 10 months, the amplitudes of cone ERG's, evoked by flashes on an adapting background, were reduced 30-60%. Both A-wave and B-wave amplitudes were significantly smaller than in either control group. Rod-dominated ERG's were not affected. At 18 months of age, well past infancy in this species, taurine levels were still reduced by 35% in plasma and by 50% in biopsied muscle tissue. Cone ERG amplitudes were no longer significantly reduced.

In adult rhesus monkeys, a taurine-free semipurified diet produced no plasma or tissue taurine depletion and no ERG deficit, even when fed for several years. However, taurine depletion was produced by a diet which, in addition to lacking taurine, was low in total protein and therefore in the sulfur-containing amino acid precursors for taurine synthesis. Plasma taurine levels of adults fed this diet were reduced 75-80% and their cone ERG amplitudes were reduced 60-70%. Ophthalmoscopic examination did not detect changes related to taurine depletion. Electron microscopy revealed abnormal swelling of cone pedicles.

These data suggest that: 1. Functionally significant taurine deficiency occurs in rhesus monkeys; 2. The deficiency is characterized by specific impairment of cone function; 3. Vulnerability to dietary taurine depletion persists at least throughout infancy; 4. Unlike cats, which show subnormal ERG's only when plasma taurine levels fall below 5% of normal, monkeys show ERG deficits with plasma depletion of only 50%, the same degree of depletion seen in human newborns fed commercial infant formulas.
Supported by HD-11129 to J.A. Sturman.

50.18 IN UTERO EXPOSURE TO LEAD ACETATE IN THE RAT: ASSOCIATED IMPAIRMENT OF CEREBROSPINAL FLUID DYNAMICS IN EARLY POST-NATAL DEVELOPMENT. J.D. Mann, J.D. Charlton*, N.E. Pederson* and R.N. Johnson. Dept. of Neurology and Biomedical Engineering, University of North Carolina Sch. of Med., Chapel Hill, NC 27514

We have previously demonstrated delayed maturation of the cerebrospinal fluid (CSF) flow system in rabbits exposed to lead during the neonatal period (Mann et al., Soc. Neurosci. Abstr. 6:829, 1980). In the present study we tested the hypothesis that in utero exposure to lead is associated with impaired CSF dynamics in early post-natal development. Pregnant albino rats were given either lead acetate (30 mg/kg) or sodium acetate by tail vein on day 16 of gestation. Litter size was reduced to six at the time of delivery. CSF dynamics were tested at 40 or 70 post-natal days in different groups using a previously described technique, (Mann et al., Ann. Neurol. 3:156-165, 1978). Under halothane anesthesia (1% in 0_2), artificial CSF was infused into the cisternal subarachnoid space at a series of constant rates until steady state intracranial pressure was achieved for each infusion rate. A mathematical model of CSF dynamics was used in data analysis for determination of resistance to absorption of CSF, intracranial compliance, and rate of CSF formation. Brain water content was determined at the time of sacrifice. Brain and blood lead content was measured by atomic absorption spectroscopy.

The incidence of ventricular enlargement was 15% in the lead exposed animals compared to 0.8% in controls. Brain water content, and brain and blood lead levels were not different between lead exposed and control groups at either age tested. Resting intracranial pressure was also unaffected by prior exposure to lead. At 40 days of age, maximum resistance to absorption of CSF was increased by 59.8 ± 15.8% (S.E.) in lead exposed animals compared to controls ($p < .02$). Rate of CSF formation at resting pressure was reduced by 29.8 ± 8% in lead exposed animals ($p < .05$). In 70 day old rats there was no significant difference in CSF dynamics when control and lead exposed animals were compared using halothane anesthesia. However, when ketamine hydrochloride (15 mg/kg) was added to the anesthetic regimen, atypical changes occurred in lead exposed animals reflecting deterioration in intracranial pressure regulation.

Hence, in utero exposure to lead on day 16 of gestation in the rat is associated with an increased incidence of ventricular enlargement and abnormal CSF dynamics. The changes in dynamics noted at 40 days are much less evident by 70 days of age.
Supported by NIH Grant HD 1443.

50.19 DEVELOPMENT OF THE VISUAL EVOKED RESPONSE IN RATS CHRONICALLY EXPOSED TO A HIGH VOLTAGE 60-HZ ELECTRIC FIELD. R. A. Jaffe, R. D. Phillips, C. A. Lopresti* and D. B. Carr*, Neuroscience Group, Biology Dept. and Energy Systems Dept., Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, WA 99352.

Electric fields approximating those generated in the vicinity of some high voltage ac power transmission lines have been shown to affect a variety of nervous system functions in the adult rat including synaptic transmission (Jaffe et al., Bioelectromagnetics 1:131, 1980) and circadian rhythms (Wilson et al., Bioelectromagnetics 2:371, 1981). Persinger et al. (Percept. Mot. Skills 36:1131, 1973) demonstrated that perinatal exposure to an electromagnetic field is capable of subsequently affecting normal rat behavior patterns. The visual evoked response (VER) has been used successfully to detect the subtle effects of various treatments including perinatal malnutrition and low level maternal lead consumption. Thus, the VER seemed well suited for detecting the possibly subtle effects of 60-Hz electric field exposure on the developing rat nervous system.

Two independent series of experiments were performed on 114 male Sprague-Dawley derived albino rat pups, representing 61 litters for experiment 1, and 53 litters for experiment 2. Animals were exposed for 20 hr/day from conception to testing (post-natal days 11-20) to a vertical 65 kV/m, 60-Hz electric field. Sham-exposed animals were housed under identical conditions without the electric field. Each rat pup was anesthetized with urethan (1.1 mg/g, ip) and mounted in a modified sterotoxic frame. Rectal temperature was maintained between 37-39°C. Recordings of the VER were obtained using a small silver ball electrode placed epidurally over the visual cortex. The reference electrode was located in the region of the nasal sinus, ipsilateral to the active electrode. Individual recording sessions began ∿25 min after anesthetic administration and 1-5 hrs after the final electric field or sham exposure. Visual stimuli consisted of 10 μsec light flashes (Grass PS-22, intensity = 16, distance = 15 cm) delivered at 0.2 Hz. Averaged VERs were obtained using a signal-averaging computer with the digitized, averaged response stored on magnetic tape. An averaged VER consisted of 64 responses sampled at 1000 Hz (1 msec/point) for 1024 msec, starting 50 msec before the flash presentation. Three such sets of averaged responses were obtained from each animal. Power spectral analysis (FFT) was performed on the tapered (split cosine-bell window) averaged VER.

The expected age-related changes were clearly evident; however, a detailed analysis of VER component latencies, peak-to-peak amplitude and power spectra failed to reveal any consistent, statistically significant effect of 60-Hz electric field exposure.
This study was supported by the U.S. Department of Energy under contract number DE-AC06-76RLO-1830.

50.20 EFFECTS OF PRENATAL CANNABINOID EXPOSURE ON BRAIN AMINES AND TESTIS FUNCTION IN MALE MICE. S. Dalterio,* R. Steger,* A. Bartke* and D. Mayfield.* (SPON: K. Blum) Depts. of Pharmacology, Anatomy & Obstetrics/Gynecology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, 78284.

Marihuana and its purified constituents have been shown to influence developmental processes in laboratory animals. The present experiments were conducted to further characterize the consequences of prenatal exposure to the major psychoactive, Δ^9-tetrahydrocannabinol (THC), or the non-psychoactives, cannabinol (CBN) or cannabidiol (CBD), to determine whether critical time periods exist for these effects and to determine their mechanisms of action. Pregnant female mice received a single oral dose (50 mg/kg) of either THC, CBN or CBD or sesame oil, 1-4 days prior to parturition. Hypothalamic and whole brain concentrations of norepinephrine (NE), dopamine (DA), serotonin (5-HT) and its metabolite 5-HIAA were measured using HPLC and electrochemical determinations in adults 2 days post-castration. Maternal exposure to CBD 2 or 4 days before parturition significantly ($P<0.01$) depressed (∿40%) NE levels in whole brain in offspring, while CBN exposure on day 1 or 3 reduced whole brain NE by 44%. Hypothalamic NE was depressed 47% only in animals exposed to CBN the day prior to birth. A marked increase ($P<0.01$) in 5-HT content was observed in hypothalamus (70% in CBD and 87% in CBN-treated animals) and in whole brain (66% in CBD and 94% in CBN-treated animals) regardless of exposure day. Hypothalamic 5-HIAA was increased 40% in CBN and 36% in CBD-exposed animals, and in whole brain 42% and 45% by CBN and CBD, respectively. Exposure to CBN and CBD also reduced whole brain DA levels by 35% and 38%, respectively, while hypothalamic DA was reduced significantly (46%) only in those animals exposed to CBN 2 days before birth. Concentrations of brain amines were the same in oil-treated (control) mice, regardless of prenatal treatment day. Seminal vesicles weights were increased by CBD exposure at least 3 days before birth, but were reduced by CBN at these times (308 ± 12/CBD; 164 ± 16/CBN vs 258 ± 13/oil mg; $P<0.05$). Testis weights were not affected, but testicular testosterone levels were reduced after exposure to CBN at 3 or more days before birth (69 ± 17 vs 138 ± 22 ng/ml, $P<0.05$). In this study, a single prenatal THC exposure had no effect. However, in our previous reports perinatal THC exposure affected body weight regulation, pituitary-gonadal function, responsivity of vas deferens to NE in vitro and adult sexual behavior. Therefore, it is evident that (1) both psychoactive and non-psychoactive cannabinoids influence developmental processes; (2) time and/or duration of exposure may be a critical factor; (3) alterations in brain amines may be related to the disruption of reproductive function produced by perinatal cannabinoid exposure. (Supported by NIDA grant DA 02342.)

50.21 EFFECTS OF CAFFEINE ON BEHAVIORAL STATE DEVELOPMENT IN THE NEWBORN RABBIT, L.P. Zeidner*, V.H. Denenberg, E.B. Thoman, P. Kramer*, J. Rowe*, A. Philipps*, J. Raye*. Dept. of Biobehavioral Science and Dept. of Neonatology, University of Connecticut, Storrs and Farmington, CT 06268.

Recent studies in our laboratory (Denenberg et al.,1982 ; Thoman et al., unpublished) have indicated that theophylline, a central respiratory stimulant in common therapeutic usage for control of apnea in premature infants, results in prolonged behavioral state disturbance in both the newborn rabbit and the premature infant. Since the infant rabbit, unlike the human infant, does not convert theophylline to caffeine, the question arises as to the generalizability of these findings to other related methylxanthines. That was the purpose of this study. On Day 1 of life (Day 0 = birth), the sleep-wake behavioral states of rabbits were measured for 2 hr., after which half the animals received 10mg/kg of caffeine by intubation (N=12), whereas the remainder received normal saline (N=12). Behavioral states were then measured for 2 hr. periods on Days 2,3,5,7,15,20,30, and 40. Caffeine sharply reduced active sleep starting on Day 2 and continuing through Day 40. The development of quiet sleep was delayed for 10 days in the drug-treated group. Paralleling the loss of active sleep was a major increase in wake between Days 2 and 40. The drug also affected the intermediate states of sleep-wake transition and active-quiet sleep transition. The data closely parallel our previous results with theophylline and show even more prolonged effects, raising further questions about the therapeutic safety of the methylxanthines in the young organism and the implications of dietary intake of caffeine by the gestating and lactating mother.

50.PO BRAIN DEVELOPMENT AND LITTER SIZE. Stephen Zamenhof and Edith van Marthens*. Mental Retardation Research Center and Brain Research Institute, UCLA School of Medicine, Los Angeles, California 90024.

In the previous work we have demonstrated that prenatal operative restriction of litter size (number in the litter) leads to newborns with increased values of brain parameters. (van Marthens and Zamenhof, Exper. Neurol. 23, 214, 1969).

The present work is a study of the effect of natural litter size (range 2 to 17) on the brain parameters of individual newborns, as well as on the "brain mass" (total of brain weights in a litter). The study was performed on 2725 newborn rats. It was found that brain weight, brain DNA content (index of cell number) and brain protein content (potential for postnatal brain development) were significantly negatively correlated with litter size. ($r = -0.382$; $p < 0.005$; $r = -0.127$, $0.01 < p < 0.025$; $r = -0.311$, $p < 0.0005$, respectively). The highest brain weights in each litter (means for all litters of the same litter size) were also significantly negatively correlated with litter size ($r = -0.296$; $p < 0.0005$). The ratio of brain protein/brain DNA (index of brain cell size) also showed such a correlation ($r = -0.226$; $p < 0.001$). However, the ratio of brain weight/body weight showed a minimum at the mean litter size (9.45; optimum for this strain?) and increased towards lower and towards higher litter sizes. The total neonatal brain masses per litter, the ratios of brain mass/maternal weight at conception, and the ratios of brain mass/average maternal food consumption per 24 hrs, increased linearly with litter size. It is concluded that: 1. Neonatal brain weight, brain cell number, brain protein and brain cell size are the higher, the smaller is the natural litter size. 2. The highest values of neonatal brain weights in each litter are also more favorable when the litter sizes are low. 3. However, the total brain mass produced per litter, and the brain mass produced per food consumed, are more favorable when the litter size is high. (Supported by NIH Grant AG-00162).

51.1 GROWTH FROM REGENERATING GOLDFISH RETINAL CULTURES IN THE ABSENCE OF SERUM OR HORMONAL SUPPLEMENTS: TISSUE EXTRACT EFFECTS. James E. Johnson and James E. Turner, Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

Goldfish retinal cultures have been established as a model for studies of optic nerve regeneration in vitro. Like other neuronal culture preparations, explanted or dissociated retina cultures have required serum or hormonal substitutes for survival and minimal neurite outgrowth in vitro. To determine the minimal requirements for regeneration in culture and to examine the effect of putative endogenous neurotrophic factors from local and target tissues, cultures were prepared in media without serum or defined hormonal supplements. Retinas chopped into 500 μ^2 explants were grown on a polyornithine substrate in Leibovitz (L-15) media with 20 mM HEPES and gentomycin sulfate. Media were supplemented further with either 1) 0.6% methyl cellulose (Dow), 2) 10% fetal calf serum (FCS) (GIBCO) or 3) cellulose and FCS. A dissociated cell preparation was developed to determine the minimal conditions for retinal ganglion cell survival and outgrowth. Retinas treated with 0.1% hyaluronidase and dissociated without serum in 0.1% Pronase and 0.1% DNase were grown under identical conditions. Results indicate that explants taken from retinas 14 days after a prior optic nerve crush are capable of survival and minimal neurite outgrowth in the absence of serum or defined hormonal supplements when grown with methyl cellulose or when dishes are preplated with methyl cellulose. Cultures grown with serum and cellulose showed a 2.5 x increase in the length of outgrowth over cellulose alone. A 2.5 x increase is also seen when explants are grown in media supplemented with cellulose and a crude extract prepared from the target optic tectum. Extracts taken from other brain regions with equal protein concentrations showed only a 1.5 X increase in length. Results from dissociated retinal cultures indicate that individual ganglion cells, unlike the intact explant, do not regenerate outgrowth in the absence of serum or hormonal supplements. Taken together these results suggest a possible role of local endogenous neurotrophic factors in a model of successful central nervous system regeneration.

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51.2 ABSENCE OF STABLE RETINA-MUSCLE SYNAPSES IS RELATED TO ABSENCE OF ACETYLCHOLINE RECEPTOR AGGREGATION FACTOR IN RETINA NEURONS. Jeffrey M. Thompson & Stanley I. Rapoport. Lab. of Neurosciences, Gerontology Res. Ctr. Natl. Inst. on Aging, NIH, Balto. MD 21224.

Chick embryo retina neurons are capable of forming synapses on rat muscle cells in vitro (Puro et al., PNAS, 74:4977-4981, 1977; Ruffolo et al., PNAS, 75: 2281-2285, 1978). These inappropriate synapses are transient and terminate within 7 days in culture. In contrast, chick embryo spinal cord neurons form stable or long-lived synapses on rat muscle cells which remain for over two weeks (Ruffolo et al., PNAS, 75: 2281-2285, 1978; Thompson et al., Develop. Neurosci., in press, 1982). Spinal cord neurons possess a factor which causes aggregation of muscle acetylcholine receptors (AChR) into high density clusters (Podleski et al., PNAS, 75: 2035-2039, 1978; Jessell et al., PNAS, 76: 5397-5401, 1979; Schaffner & Daniels, J. Neurosci., in press, 1982). This study investigated whether retina neurons also possess this factor.

Neural conditioned medium and neuron-muscle cocultures using chick embryo retina or spinal cord neurons were examined for their effect on induction of AChR clusters. Conditioned medium (CM) was prepared by collecting medium from cultures of retina neurons (20 x 10^6 cells/35 mm dish) or spinal cord neurons (5 x 10^6 cells/35 mm dish) on days 2 and 3 after plating, and added to rat myotube cultures (7-10 days after plating) for 24 hr. Cocultures of neurons and muscles were prepared by plating 20 x 10^6 retinal neurons from 8-day chick embryos or 5 x 10^6 spinal cord neurons from 6-day chick embryos on rat myotube cultures. AChR clusters were determined after 24 hr in conditioned medium treatments, and after 1, 3 and 5 days in cocultures. AChR clusters were visualized with tetramethylrhodamine α-bungarotoxin (gift of Z. Vogel and M. Daniels). 46-138 myotubes were counted per dish. The number of clusters in CM treated myotube cultures was: control - 0.52 ± 0.04 clusters (mean ± S.E.M.); retina CM - 0.49 ± 0.03 clusters; spinal cord CM - 0.74 ± 0.04 clusters (N = 9 experiments). The number of clusters in cocultures was: Day 1: control - 0.59 ± .20; retina - 0.53 ± .13; spinal cord - 1.41 ± .06; Day 3: control - 0.66 ± .18; retina - 0.88 ± .28; spinal cord - 3.10 ± .69; Day 5: control - 0.40 ± .15; retina - 0.59 ± .06; spinal cord - 3.27 ± .06 (N = 3 experiments). The increase in clusters using spinal cord CM or in spinal cord cocultures was significant ($p < 0.01$).

The results indicate that while spinal cord neurons possess a factor which enhances clusters of AChR on culture myotubes, retina neurons do not possess such a factor. The results were similar in either CM of cocultures, although cocultures were more dramatic. The absence of an aggregating factor in retina may relate to the lack of stabilization of retina-muscle synapses, as opposed to the stabilization of spinal-cord muscle synapses.

51.3 RESOLUTION OF THE DENERVATION-DEPENDENT PHOSPHOPOLYPEPTIDE IN RAT SKELETAL MUSCLE CYTOSOL. J.A. McLane, S.P. Squinto* and I.R. Held. Neuroscience Research Laboratory, VA Hospital, Hines, IL 60141 and Loyola University Medical Center, Maywood, IL 60153.

Motor neurons influence the muscle cells which they innervate not only by propagation of nerve impulses, but also through trophic actions. Fast axoplasmic transport of substances from their sites of synthesis in neuronal perikarya to the neuronal endings provides a credible mechanism for trophic regulation of muscle. We have shown that within hours after denervation there is an increase in the in vitro phosphorylation of an endogenous cytosolic polypeptide(s) which is temporally correlated with the length of the distal nerve stump remaining attached to the muscle (Trans. Am. Soc. Neurochem. 12:230, 1981). A single major ^{32}P-labeled cytosolic protein has been resolved from in vitro assay mixtures by SDS polyacrylamide gel electrophoresis on cylindrical rods and by exclusion chromatography on Sephadex G-150 or a Waters I-125 HPLC column (Soc. Neurosci. Abstr. 7:931, 1981). We have obtained greater resolution of the cytosolic components of the in vitro assay mixture on SDS slab gels containing a 5-15% acrylamide gradient. With this gel system 45 Coomassie blue staining peptides are reproducibly resolved with ten ^{32}P-labeled polypeptides easily detectable by autoradiography on x-ray film. The major ^{32}P-labeled polypeptide has a relative molecular weight in this gel system of 56K and contains greater than 80% of the total radioactivity. Other, more weakly radiolabeled polypeptides of interest are found to have molecular weights of 47K, 45K and 18K. When radiolabeled cytosol samples from denervated muscles showing maximal increases in cytosolic phosphorylating activity are resolved on slab gels, an increased phosphorylation is found in the major phosphoprotein. Addition of cyclic AMP-dependent protein kinase catalytic subunit to the in vitro assay results in a marked increase in radiolabeling of many polypeptides, especially in the area of the 47K, 45K and 18K proteins, but only a slight increase in $^{32}PO_4$ incorporation into the 56K polypeptide. Addition of 10^{-7} to 10^{-5}M cyclic AMP to in vitro assays results in only a slight increase in total $^{32}PO_4$ incorporation, but when resolved on the SDS slab gel the radiolabeling of several minor phosphopolypeptides is increased and the labeling of the denervation-dependent, 56K phosphoprotein is dramatically reduced. These results and preliminary studies utilizing 8-azidoadenosine 3':5'-cyclic monophosphate, a photoaffinity analogue of cyclic AMP suggest that the 56K phosphoprotein may be the regulatory subunit of the type II cyclic AMP-dependent protein kinase.

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51.4 NEWLY INSERTED ACH RECEPTORS AT THE NEUROMUSCULAR JUNCTION HAVE A FAST RATE OF DEGRADATION. E.F. Stanley, D.B. Drachman. Johns Hopkins University, School of Medicine, Baltimore, Maryland.

It is generally believed that acetylcholine receptors (AChRs) at the intact neuromuscular junction constitute a homogeneous population with a single slow turnover rate. However, different studies have reported AChR half lives ranging from 6 days (Berg and Hall, 1975) to 13 days (Linden and Fambrough, 1979). We have therefore tested the hypothesis that the turnover rate of junctional AChRs may not be uniform, by comparing the degradation rates of "new" (< 6 days) and "old" (> 6 days) AChRs.

AChRs of mouse diaphragms were labeled in vivo with ^{125}I-α-BuTx, and the loss of radioactivity from the diaphragms of groups of mice killed serially at 2 day intervals for up to 12 days was used to determine AChR degradation rates. "New" AChRs or "old" AChRs were labeled in separate sets of mice: "New" AChRs were labeled by first blocking existing AChRs with non-radioactive α-BuTx, and 6 days later labeling with ^{125}I-α-BuTx. "Old" AChRs were labeled with ^{125}I-α-BuTx on day 0, and their rate of loss was followed from day 6 on.

"New" AChRs were degraded with a half life of 3.6 ± 0.4 days (4 experiments) while "old" AChRs had a much slower half life of 10.0 ± 0.8 days (3 experiments).

Thus, AChRs at the intact neuromuscular junction do not constitute a single homogeneous population. The receptors that have been newly inserted into the membrane are degraded at a faster rate than those that have survived for longer periods. The co-existence of AChRs with slow and fast degradation rates raises two intriguing possibilities: Either there are two or more separable pools of AChRs with intrinsically different degradation rates, or the transition from a fast degradation rate to a slow degradation rate reflects the stabilization of AChRs on the postsynaptic membrane.

51.5 TROPHIC REGULATION OF MUSCLE ENZYME, Ca++ AND cGMP CONTENT BY NERVE. C. L. Weill. Depts. of Neurology and Anatomy, Louisiana State Univ. Med. Ctr., New Orleans, LA 70112.

The trophic phenomena associated with nerve-muscle interactions have been extensively studied, but their molecular foundations have yet to be determined. I present here studies designed to describe in detail and determine the molecular foundations for the trophic effects of nerve on muscle during development.

The specific activities of acetylcholinesterase (AChE), creatine kinase (CK), aldolase (ALD) and lactate dehydrogenase (LDH) were determined on homogenates of cultured chick muscle maintained in the presence and absence of 50% spinal cord-conditioned medium (SC-CM) for 9 days. Relative to controls, the activities of CK, ALD and LDH were 1.3, 1.5 and 2.1-fold higher respectively on day one. By day 5 the activities had declined to 64, 35 and 52% of controls respectively. The mean reduction in activity from day 1 to day 5 was 2.8-fold for CK, 1.5-fold for LDH and 2.5-fold for ALD. In contrast AChE was 20% lower than controls on day 1, and 1.24-fold higher on day 5. Over days 1-5 cellular AChE activity displayed an increase of 1.5-fold. Over 9 days there was no significant difference in total cellular protein of controls relative to SC-CM treated cultures.

The rate of secretion of each enzyme was also measured over 9 days on the same cultures. The rates qualitatively reflected the total cellular content; for CK, ALD and LDH they declined over 9 days while the rate for AChE increased over the same period. The secretion rate for total protein displayed a 1.8-fold pulse increase of 24-36 hr duration on 5 relative to controls.

Calcium influx rates were measured on sister cultures over 8-9 days and found to undergo a mean increase from day 1-8 of 3.8 $\pm$ 1.3-fold. The day 1 values were 58 $\pm$ 14% of controls, while the day 8-9 values were 3.3-fold greater. Total Ca++ uptake was comparable to controls through day 4-5 after which it declined to 38% of control by day 8-9.

The cGMP content of sister cultures was determined by RIA. Control cultures displayed little change in cGMP content over 7 days in culture. SC-CM treated cultures displayed similar levels except during a 24-36 hr period around day 5 when the cGMP rose 2.35 $\pm$ 0.49 (m $\pm$ S.E.)-fold. By day 6 it had declined and remained at control levels.

In conclusion, cultured chick spinal cord neurons secrete factors that alter several functions of cultured muscle; a) enzyme content was altered differentially, CK, ALD and LDH down regulated and AChE up regulated, b) calcium influx was depressed and then increased and c) temporally coincident with the increase in calcium influx rate and a pulse increase in total protein secretion, cellular cGMP displayed a pulse increase of similar duration. The causal relationships between these parameters is under study.

51.6 AN EXTRACELLULAR MATRIX FACTOR THAT ORGANIZES ACETYLCHOLINE RECEPTORS. Earl W. Godfrey*, Ralph M. Nitkin, Bruce G. Wallace and U.J. McMahan, Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

An extracellular matrix preparation (ECM) from the electric organ of Torpedo californica is rich in a factor(s) that organizes the acetylcholine receptors (AChRs) on cultured chick myotubes into distinct clusters (Rubin and McMahan, Soc. Neurosci. Abstr. 6:330, 1980). This factor is of particular interest because the clustering of AChRs at regenerating neuromuscular junctions in vivo may also be directed by components of extracellular matrix (Burden et al., J. Cell Biol. 82:412, 1979). The studies reported here are aimed at identifying the Torpedo AChR organizing factor and learning whether there is a similar molecule at the neuromuscular junction. We have solubilized the Torpedo factor by treating ECM with high salt, have made an antiserum against the high salt extract that binds both to the Torpedo factor and to extracellular matrix at frog neuromuscular junctions, and are now purifying the factor with a view to making a specific antiserum.

The factor influences the organization of AChRs in the following way. As the amount of extract added to a culture is increased the number of receptor clusters per myotube increases and plateaus at a level 3- to 10-fold higher than in control cultures. The response is maximum within 16 hr. The factor causes AChRs in the muscle cell plasma membrane to migrate laterally into clusters; it does not cause an increase in the number of AChRs on the cell surface.

Antiserum was made by injecting high salt extract of ECM into rabbits. The antiserum bound to the factor and blocked its active site. Frog cutaneous pectoris muscles from which nerves and muscle cells had been removed were incubated in the same antiserum and binding sites were examined by light and electron microscopy after immunocytochemical staining. The extracellular matrix of the muscle stained and the stain was concentrated at the former sites of neuromuscular junctions.

To date we have purified the factor about 1000 fold by a sequence of gel filtration, ion exchange chromatography on DEAE-cellulose, and affinity chromatography using Concanavalin A-Sepharose. The activity elutes as an acidic glycoprotein with a broad molecular weight distribution of 60 to 100 kd and has an isoelectric point of pH 4 to 5 as determined by isoelectric focusing.

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51.7 AXONS WITH MAJOR TROPHIC DEPENDENCIES ON AXONAL TRANSPORT OR AXON-SHEATH GLIA SHOW DIFFERENCES IN CONCENTRATION OF AXOPLASMIC MEMBRANOUS ORGANELLES. K.R. Seshan*, G.D. Bittner, T.A. Viancour, and M.A. Raymond*. Zoology , Univ. Texas, Austin, TX 78712.

Axoplasmic transport of proteins and other substances synthesized in neuronal somata appears to be a universal phenomenon in nervous systems, and an accepted mechanism for the trophic support of axonal chemical synapses and plasma membranes. Axon-sheath glia also contribute to axonal maintenance in at least some species. Smooth endoplasmic reticulum, micro- and neuro-tubules are the apparent morphological substrates for axoplasmic transport. Exo- and endo-cytotic vesicles are implicated in glia to axon transport. Axoplasmic concentrations and distributions of these membranous organelles should therefore reflect an axon's relative dependence on a particular trophic mechanism.

To investigate this possibility, we have examined two identified axons reported to have different trophic dependencies (see Bittner, Comp. Biochem. Physiol., 68A, 299-306 (1981)). The medial and lateral giant fiber systems of crayfish mediate complementary tail-flip escape behaviors, and appear to make efferent synapses of essentially equivalent number, strength and type. The medial giant axon (MGA) is a single, continuous fiber which terminates in the 6th abdominal ganglion at least 50mm (in adults) from its cell body located in the supraesophogeal ganglion. The lateral giant fiber, however, arises from segmental neurons whose giant axons (LGAs) form septate, electrical junctions which permit uninterrupted spike conduction. In adults, an LGA is typically no more than 5mm from its cell body. Isolated abdominal segments of MGAs survive physiologically and morphologically intact for months, whereas equivalent segments of LGAs degenerate completely within a few days. These and other data suggest that an MGA derives substantial trophic support from its sheath glia, whereas an LGA depends primarily on axoplasmic transport of substances from its cell body.

We have examined cross-sections of LGAs and MGAs in the abdominal nerve cord of P. clarkii. Viewed with a light microscope, basic dyes such as toluidine blue consistently stain LGA axoplasm more densely than MGA axoplasm. Ultrastructurally, the concentration of neurotubules in LGAs is twice that of MGAs, and LGAs contain a much more extensive smooth endoplasmic reticulum. In both axons, smooth endoplasmic reticulum is densest near the axon membrane. Relative concentrations and distributions of vesicles are being determined at the time of this abstract. However, our initial data are consistent with the hypothesis that relative concentrations and distributions of intracellular organelles can reflect different trophic dependencies.

51.8 BIOCHEMICAL SEPARATION OF TARGET- AND SUBSTRATE-MEDIATED RESPONSES OF DEVELOPING SPINAL CORD EXPLANTS. W. L. Muhlach* and E. D. Pollack (SPON: R. Ruth). Inst. Study Develop. Disabil., University of Illinois, Chicago, IL 60608.

The quantity, rate and direction of neuritic outgrowth from developing tadpole spinal cord explants have been shown to be under stage-specific control of their developmental target, i.e. limb mesenchyme (Pollack and Muhlach, Dev. Biol.,'81). Biochemical assays were performed on tissue grown under varying experimental conditions to further elucidate the mechanisms involved in neural tissue response to target influence. The protein content (standardized to DNA) of stage V spinal cord tissue (Taylor-Kollros stages), co-cultured with target tissue (stage V limb) on collagen substrate, was dramatically increased by 52% over control levels (spinal cord cultured alone). Cultures of spinal cord on poly-DL-lysine (PLYS) substrate resulted in a 32% increase in protein content in the absence of target and a 41% increase when target was present. These protein levels correspond to the increases found in neuritic outgrowth under these same conditions. Choline acetyltransferase (CAT) activity was similarly increased over control levels in the presence of target on collagen (29.6%) and on PLYS (33.9%) but was only slightly elevated on PLYS in the absence of target (9.7%). Cultures of spinal cord with skeletal muscle of older tadpoles (st. X-XII) produced a 24.5% increase in protein on both collagen and PLYS but no increase in CAT activity on either substrate. The presence of limb tissue also increased the amount of DNA/culture (by 16.6%) over controls. This increase relates to the motor neuron survival influences of the limb (Pollack, Neurosci. Abstr.,'82). Since the PLYS substrate can mimic the effect of target tissue on protein synthesis by the cord explant, it appears that the target's action in this instance may be a substrate mediated process. However, PLYS alone does not mimic the effect of target on increased CAT activity in the spinal cord tissue. A similar situation occurs in the effect of muscle on protein content (increased), but not on CAT activity of the spinal cord. Thus, it seems that there are several cellular components of the spinal cord response to target tissue: 1) a substrate associated mechanism, probably involving neurite-substratum adhesion, that results in increased protein production and corresponding enhanced neuritic outgrowth; 2) stimulation of functional maturation and other metabolic machinery as those involved in neurite growth rates (Muhlach and Pollack, Dev. Brain Res.,'82) or CAT activity that are not directly associated with the substrate; 3) increased survival of spinal cord cells. (Supported by NIH grant NS 13814.)

51.9 SEPTAL LESIONS INCREASE CHELATABLE ZINC IN MOSSY-FIBER REGION. G.R. Stewart*, F.W. Gage, G.A. Howell and C.J. Frederickson
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A unique pool of chelatable zinc associated with the hippocampal mossy fibers has been demonstrated repeatedly, but the neurobiological function of that zinc remains unknown. One approach to the problem of function is to determine the responsiveness of the zinc pool to manipulations of hippocampal tissue. In the present work, we have partially deafferented the hippocampus (by septal lesions) and searched for post-lesion changes in the concentration of mossy-fiber-related zinc.

Twenty-five adult, male Sprague-Dawley rats received bilateral medial septum ablations and were sacrificed from 3 to 28 days thereafter for hippocampal zinc assays; 8 additional animals served as unoperated controls. Zinc was assayed by the zinc dithizonate densitometric method of Frederickson et al. (Exp. Neurol., 1981, 73:812). Briefly, dithizone was injected i.p., 15 min were allowed for the formation of the zinc dithizonate chelate in the hippocampus, and the animal was then sacrificed, the brain removed and cut frozen (100 μm), and the density of zinc dithizonate stain in hippocampal sections measured spectrophotometrically in the light microscope. For statistical analysis, all measurements from the mid-hilar region of a single, standard section of dorsal hippocampus were averaged together for each animal, yielding a single value for the intensity of zinc dithizonate staining.

Septal lesions caused a transient increase in zinc dithizonate in the hilus which was maximal (33% increase over control) at 10 days post-lesion and was virtually gone by 21-28 days. Average optical density of zinc dithizonate in the hilus was: 0.373 for controls (n=8), 0.445 at 3 days post-lesion (n=4), 0.498 at 10 days (n=6), 0.453 at 16 days (n=3), 0.392 at 21 days (n=6), and 0.418 at 28 days (n=6) ($p < .025$, 1-way ANOVA; 10-day group exceeds control at $p < .025$ by Dunnett's t statistic).

Whether the observed increase in zinc dithizonate staining reflects an increase in total zinc or an increase in the proportion of zinc which can be chelated is under examination. In either case, the results indicate that mossy-fiber zinc is a labile pool which is dynamically responsive to alterations in hippocampal innervation. Of particular interest is that the time course of the observed change in zinc parallels that of the sympathetic ingrowth occuring after medial septal lesions. Perhaps zinc metalloenzymes are directly involved in regulation of trophic phenomena in the hippocampus, as has been suggested by Crutcher and Davis (Soc. Neurosci. Abs., 1980,6:172.)

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51.10 DEVELOPMENTAL ALTERATIONS IN THE PUTAMEN OF FETAL RABBITS DUE TO CHRONIC RESERPINE ADMINISTRATION. V. M. Tennyson, M. Schoenebeck* and P. Gershon*. Depts. of Anatomy & Cell Biology and Pathology (Neuropathology), Columbia Univ., College of Physicians and Surgeons, New York, N.Y. 10032.

During development, nigrostriatal neuroblasts and axons exhibit dopamine (DA) induced fluorescence before their axons have extended beyond the vicinity of the cell bodies. In order to investigate possible developmental effects of growing nigrostriatal axons on the basal ganglia, we repeatedly gave reserpine (0.03-0.14 mg/kg/day) to pregnant rabbits to deplete DA chronically in their fetuses during critical periods of the development of the nigrostriatal system. Reserpine crossed the placenta and depleted fluorescence markedly in the fetal putamen. We showed by electron microscopy (Brain Res Bull 8, No 5/6, 1982) that reserpine treatment caused a marked reduction in the numbers of mature axonal boutons in the putamen (to less than 1/2 that of controls, $p < 0.001$), and the area occupied by growth cones in the reserpine-treated fetuses was twice that of controls ($p < 0.005$). This suggested that the neuropil of the putamen of reserpine-treated fetuses was less mature than that of controls. In this study, we wished to determine whether the decrease in striatal terminals due to reserpine treatment included dopaminergic boutons. We measured the uptake of ^{3}H-DA (a process not antagonized by reserpine). Uptake of ^{3}H-DA was decreased in the putamen of reserpine-treated fetuses to 70% of controls ($p < 0.001$). To determine whether the cell bodies of neuroblasts of the putamen exhibited differences in maturity between control and experimental fetuses, the number of cells was counted and their size estimated by point count planimetry. In control fetuses, there were 3,226 ± 55.8 SE/mm^2 and in sections they occupied an average area of 79.6 ± 0.9 SE /um^2, whereas in the reserpine-treated fetuses, there were 23% more neuroblasts (4,144 ± 92.6 SE/mm^2, $p < 0.001$), but they were 22% smaller (61.2 ± 1.3 SE/um^2, $p < 0.001$). Compared to controls, reserpine-treated neuroblasts were smaller and more numerous per unit area, and thus, less mature. They closely resembled in number and size those cells in the control that had recently migrated away from the subependymal cell plate. A small number of the giant type of neuroblast was also found in the control putamen (14.1 ± 0.9 SE /mm^2). This cell type was somewhat reduced in reserpine-treated fetuses (10.2 ± 0.7 SE/mm^2, $p < 0.01$). The results are consistent with the hypothesis that early stores of DA may affect the ability of dopaminergic axons to reach the striatum and result in a retarded development of postsynaptic structures; however, other non-specific effects of reserpine have not been ruled out.

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51.11 INTERCELLULAR MOLECULAR TRANSPORT: INTRA-AXONALLY INJECTED HORSERADISH PEROXIDASE RAPIDLY ENTERS AXON-SHEATH GLIA. T.A. Viancour, G.D. Bittner and K.R. Seshan*. Zoology Department, Univ. Texas, Austin, TX 78712.

Our recent report of Lucifer Yellow CH movement from an injected axon into axon-sheath glia (Viancour et al., Nature 293, 65-67 (1982)) demonstrated molecular exchange between axoplasm and gliaplasm. Because of the low molecular weight of the dye, we did not propose a specific exchange mechanism. The hydrated Lucifer ion is small enough to have diffused through cytoplasmic pores of gap junctions, or it could have been exchanged by axonal exocytosis and glial endocytosis, or it could have passed through the somewhat controversial regions of unrestricted cytoplasmic continuity between crustacean axons and glia reported by Peracchia (Nature, 290, 597-598 (1981)).

To obtain evidence for or against one or more of these mechanisms, we have iontophoretically injected the 40,000-dalton protein horseradish peroxidase (HRP) into giant axons of crayfish (P. clarkii). The large diameter of hydrated HRP should prevent diffusion through the pores of gap junctions, but not through the 15-20nm pores reported by Peracchia; if molecular exchange is by exocytosis/endocytosis then electron-dense HRP reaction product should be found in axo- and glia-plasmic vesicles. To avoid the possibility of HRP movement during or following fixation, we have precipitated the Hanker-Yates, HRP reaction product prior to fixation.

We find HRP reaction product bound to smooth endoplasmic reticula and neurotubules in the injected axon. Following injection periods of one hour or less, reaction product has been found in cytoplasmic vesicles within the glial cells immediately adjacent to the injected axon. Concentrations of such vesicles are often found adjacent to invaginations of the axon membrane.

We conclude that functionally intact, large and complex molecules such as HRP can move rapidly from a crayfish giant axon into the glial cells of its sheath. To date, our evidence favors a mechanism of molecular movement involving axonal exocytosis and glial endocytosis. Our results complement those of Nordlander et al. (J. Comp. Neur., 161, 499-514 (1975)) to provide evidence for a mechanism of rapid, two-way transport of metabolites between axons and axon-sheath glia in crustacea.

51.12 GLIAL FACTOR INDUCES DIFFERENTIATION OF NEUROBLASTOMA x GLIOMA HYBRID CELLS IN CULTURE. S.F. Atweh and B.G.W. Arnason. Dept. of Neurology, The University of Chicago, Chicago IL 60637

Neuroblastoma x glioma (NG) hybrid cells, NG-108-15, possess many neuronal properties including opiate receptors (OR) and choline acetyl transferase (CAT), that can be modulated in culture. Both parameters were shown to increase with time after plating and become maximal at confluence (Atweh, Neuroscience Abstracts, Vol. 7, #139.2,1981; Schnarr and Dahms, Neuroscience Abstracts, Vol. 7, #224.18,1981). Furthermore, there is evidence that NG cells themselves secrete a "factor" which stimulates the increase in OR and CAT. "Conditioned" media obtained from very confluent NG cells increased OR and CAT in non-confluent cells. Glia or glia-derived cells are known to secrete factors that stimulate the growth of neural cells in culture. In this study we tested the hypothesis that NG cells may respond to glial factors.

NG cells were grown in culture medium consisting of Dulbecco modified Eagle's medium and 5% fetal bovine serum. Two or three days after plating the medium was changed. Control flasks received fresh medium. Experimental flasks received "conditioned" medium that had been incubated with semi-confluent C_6 glioma cells for 24 hours, or fresh medium to which was added 1mM dibutyryl CAMP (DbCAMP). The cells were harvested 24 hrs later, counted and homogenized. OR and CAT activity were measured in the homogenate. Cells grown in C_6-conditioned medium exhibited a 55% increase in OR and a 62% increase in CAT activity as compared to controls. The C_6-conditioned medium did not alter the rate of cell division and did not induce any morphological change in the cells. DbCAMP, on the other hand, inhibited cell division and induced a morphological change in NG cells characterized by increased process formation and thickening of processes. In DbCAMP treated cultures CAT activity increased from 80-100%, but there was a 56% decrease in OR.

This data suggests that a glial derived factor(s) can induce the formation of neuronal like properties in NG cells in tissue culture and that this factor(s) has similar effects to those secreted by NG cells. Although DbCAMP induces cell differentiation, the changes induced by DbCAMP are qualitatively different from those induced by the glial "factor". It is still conceivable that some of the effects induced by the glial factor may be mediated by cyclic AMP, especially those related to CAT activation. The data also demonstrates that "growth factors" can modulate surface receptors (such as OR) as well as intracellular enzymes, and that the mechanisms underlying the modulation of receptor may be independent of those affecting intracellular enzymes.

In addition to studying some of the mechanisms underlying the regulation of neuronal functions, this system can be useful in the study of glial-neuronal interaction.

51.13 ROLE OF THE ORIGINAL SYNAPTIC SITE ON ACETYLCHOLINE RECEPTOR CLUSTERING AND NERVE-MUSCLE CONTACT FORMATION IN REGENERATING RAT SKELETAL MUSCLE. M.D. Womble*(SPON: K.F. Barald).Dept. of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109.

After regeneration of rat or frog muscle, acetylcholine receptors (AchR's) are clustered at original synaptic sites, even in the absence of nerves (Bader, J.Cell Biol.88,1981; Burden,et al., J.Cell Biol.82,1979). In the frog, with subsequent innervation, neuronal contact is found exclusively at former endplate (EP) sites (Marshall,et al.,PNAS 74,1977). Interruption of original nerve supply to non-injured rat muscle induces ectopic sites of AchR clustering and EP formation at contact points with foreign nerve terminals (Lomo & Slater, J.Physiol.303,1980). In the present study, fluorescent α-bungarotoxin binding and a silver nerve stain are used to examine the appearance of AchR clusters and the role of the original EP zone in grafts of the rat soleus muscle. Myofibers of the graft degenerate and are phagocytosed. New fibers regenerate within the surviving basal lamina tubes. Previous work (Womble, Anat.Rec.202,1982) shows, by 5 days after grafting, large EP-like clusters of AchR's appear spontaneously on newly regenerated myotubes in the central third of the muscle. As returning nerve fibers penetrate into the proximal end, new AchR clusters are induced in this region at sites of nerve-muscle contact. Current work shows that EP-like AchR clusters develop only at former synaptic sites, identified by residual acetylcholinesterase (AchE) activity. Most older grafts (up to 60 days) have nerves well into the central zone of the muscle. Nerve fibers appear to seek out and synapse exclusively with the EP-like areas, accompanied by a disappearance of proximal nerve contacts and AchR patches. In cases where nerves do not reach the central zone, the EP-like clusters are lost and proximal EP's are retained. To further test the role of the original synaptic site, the motor endplate (MEP)-containing portion of the soleus muscle was removed at the time of grafting. A previous report (Bader, Dev.Biol.77,1980) showed that as few as 2% of the normal number of EP's are found in 60 day MEP-less grafts. Preliminary results indicate that early MEP-less grafts (up to 20 days) become well innervated and numerous nerve-associated AchR clusters appear throughout the muscle. The number of these contacts begins to decline by 30 days postgrafting. Thus, the original MEP zone may be important in selectively stabilizing some transient nerve-muscle contacts which form early in the regenerative process and later disappear in MEP-less grafts, as nerves withdraw. (Supported by NIH and MDA grants to Bruce M. Carlson.)

51.14 REGULATION OF AChR CLUSTERS BY EXTERNAL CALCIUM CONCENTRATION. S. Bursztajn, J. L. McManaman and S. H. Appel. Neurology Department, Baylor College of Medicine, Houston, TX 77030.

Clustered and diffuse acetylcholine receptors (AChR) are present in cultured rat skeletal muscle. We have investigated the effect of extracellular calcium (Ca^{+2}) concentration on the distribution of surface AChR in cultured rat myotubes. We demonstrate that high CA^{+2} concentration (15 mM) leads to a significant increase in size and stability of AChR clusters without an increase in synthesis of new AChR. Six-day old cultures were incubated in Ca^{++}-free Dulbecco-Vogt modified Eagle's medium to which Ca^{++} (2.5×10^{-5}M - 1.5×10^{-2}M) was added. The final Ca^{++} concentration in the medium was measured with an atomic absorption spectrophotometer. After incubation of myotubes at various Ca^{+2} concentrations for 0-48h, cultures were stained with α-Bungarotoxin (α-BTX) conjugated to tetramethyl rhodamine (TMR), fixed in 2% formaldehyde, and clusters were visualized with the fluorescent microscope and quantitated. Cultures incubated in low Ca^{+2} (2.5×10^{-5}M) culture medium have fewer intact AChR clusters (18.5 ± 7.5%) than those incubated in high Ca^{+2} (1.5×10^{-2}M) medium (93.3 ± 3.7%). In the presence of high Ca^{+2} medium there is a time-dependent increase in the number of clusters per myotube and a two-fold increase in cluster dimensions. Furthermore, the appearance of newly formed AChR clusters following treatment with α-BTX, to block existing clusters, was also accelerated. Under identical conditions the rates of AChR synthesis and degradation are unaffected by high Ca^{+2} concentrations. The effect of Ca^{+2} on ACh receptor clusters appears to be specific for Ca^{+2} since AChR clusters were not affected by other divalent ions or incubation in hypotonic medium. High concentrations of calcium also alter the effects of myasthenic globulin (MG) on AChR cluster dispersal and turnover of surface ACh receptors. MG binds to AChR, accelerates the rate of AChR degradation and causes dispersal of AChR clusters. In the presence of MG, incubation of cells in high Ca^{+2} medium slowed the loss of intact AChR clusters and decreased the rate of AChR degradation. The decreased rate of cluster loss coincided with the decreased rate of AChR degradation. These results indicate that high concentrations of Ca^{+2} stabilize ACh receptor clusters and prevent dispersal of receptors by MG. Our results also indicate that the increased rate of AChR degradation in the presence of MG antibody may be related to the dispersal of intact AChR clusters. (Supported by MDA)

51.15 NEUROCHEMICAL CORRELATES OF HYPERREFLEXIA FOLLOWING SPINAL CORD TRANSECTION. D. J. Jones and O. F. Alcantara*. Depts. Anesthesiology and Pharmacology, The Univ. Texas Health Science Center, San Antonio, TX 78284.

Enhanced sensitivity of motor reflexes following spinal cord transection has been established in both clinical and laboratory animal investigations. Previous studies from this laboratory have established that denervation supersensitivity involving the cyclic AMP system may be a molecular component of hyperreflexia following spinal cord transection (Neurosci. Abstracts, 7:919, 1981). The present work is a description of concomitant changes in NE-containing neuron function that parallel alterations in cyclic AMP system sensitivity following spinal cord transection.

Transection of the spinal cord was performed at the midthoracic level in male Sprague/Dawley rats weighing 150-200 gm. Twenty-four hours following transection, hindlimb flexor reflexes were evaluated. Following various time periods, the flexor reflex was again measured using the same protocol. Subsequently, the animal was sacrificed and cervical (intact) and lumbar (denervated) spinal cord removed. The tissue was then processed for measurements of (a) catecholamine levels (b) NE-uptake or (c) adrenergic receptor binding. In addition, NE-stimulated cyclic AMP accumulation in tissue slices from cervical vs lumbar spinal cord was also measured.

As early as three days following spinal cord transection, spontaneous flexor reflex activity was markedly increased. In addition the amplitude of flexion (excursion) at 0.6 mA was significantly increased. This hyperreflexia is evident at least through 60 days following transection. There occurred a gradual decrease in NE uptake and in NE steady state concentrations below the level of transection such that at 21 days, greater than 95% depletion of NE existed in lumbar spinal cord.

Evidence of denervation supersensitivity was represented by a 3-4 fold greater increase in NE-stimulated cyclic AMP accumulation in lumbar vs cervical cord tissue slices from transected rats. This enhanced response was present as early as 3 days and lasts at least 90 days post-transection. Consonant with this enhanced response is an approximate doubling of the number of beta receptors (B_{max}) in spinal cord below the level of transection. There was no change in apparent K_D.

These data suggest that synaptic supersensitivity exists in lumbar spinal cord following transection and that alterations in adrenergic receptor function and number may be a part of the mediating events for reflex hyperresponsiveness following injury to the cord.

Supported by grants from the Morrison Trust Foundation and NINCDS (NS14946).

51.16 SCHWANN CELLS PROMOTE THE TRANSFORMATION OF BIPOLAR SENSORY NEURONS INTO PSEUDOUNIPOLAR NEURONS IN VITRO. A.W.MUDGE* (SPON:C.Gunderson) MRC Neuroimmunology Project, Dept. of Zoology, University College London, Gower Street, London, WC1E 6BT.

Cahal described the morphological development of chick sensory neurons which involves a remarkable rearrangement of the neuronal cytoplasm (Cahal,S.Ramon y, Trab. Lab. Invest. Biol., Univ. Madrid 3, 1904). The early neuoblasts are bipolar but as development proceeds, the two processes approach each other and the cells become bell-shaped. During the second half of development the two processes fuse giving rise to a single thick axon which bifurcates - the so-called pseudounipolar form of the sensory neuron.

Sensory neurons dissociated from 10 day old chick ganglia were plated onto collagen-coated tissue culture dishes and treated with cytosine arabinoside to kill the non-neuronal cells. To visualise the arrangement of the neuronal processes, the cultures were stained with a monoclonal antibody (RT97) raised against neurofilament proteins (Wood,J and Anderton,B, Biosci. Reports, 1, 1981), followed by an antibody to mouse IgG coupled to peroxidase: the peroxidase was reacted with diaminobenzidine/H_2O_2. 24 hours after plating the neurons were predominantly bipolar with some multipolar forms. Upto a month after plating, the neurons in the absence of other cell types retained this immature arrangement of their processes. However, when purified Schwann cells (SC) derived from rat sciatic nerve (Brockes J et al, Brain Res. 165, 1979) were added to the 'neuron-alone' cultures (at day 7 after plating) the neurons flattened out and became phase-dark. This change in shape was dependant on the number of SC added to the neurons and with high numbers of SC most of the neurons changed their morphology within 5 days of the SC addition. When such cultures were stained with anti-NF, most of the neurons had assumed the pseudounipolar form or the intermediate bell-shape form. The morphology of the neurons after the addition of fibroblasts was indistinguishable from the neuron-alone' condition.

This study provides a clear example of the ability of glial cells to support the development of neurons. It also illustrates the power of using purified populations of neurons and non-neuronal cells in order to study which properties are intrinsic to the developing neuron and which properties require interactions with other cells.

52.1 CATECHOLAMINE CONCENTRATIONS AND UPTAKE DURING SYMPATHETIC INNERVATION IN THE DEVELOPING CHICK HEART. D. E. Stewart* and M. L. Kirby (SPON: R. Holt). Dept. of Anatomy, Medical College of Georgia, Augusta GA 30912.

Developing sympathetic nerves first reach the heart of the embryonic chick on incubation days (ID) 10-11. Electrical stimulation of cardiac adrenergic nerves does not produce a positive inotropic response until ID 16. This lag between morphological and functional adrenergic transmission may be due to an inadequate density of innervation. To obtain an index of innervation during development, we have studied the ability of the adrenergic nerve terminals in the atria of the chick heart to accumulate [^{3}H]-NE throughout the incubation to 2 weeks post-hatching. Specific neuronal uptake of [^{3}H]-NE was defined as total uptake minus uptake in the presence of desmethylimipramine, a specific uptake blocker. Neuronal uptake of [^{3}H]-NE in atria (in vitro) was first observed on ID 11. [^{3}H]-NE uptake increased gradually through ID 17, reached a peak on ID 19 and then decreased until ID 21. A linear increase in uptake to maximal levels occurred during the first 2 weeks posthatching. To evaluate adrenergic regeneration capabilities and the effectiveness of 6-hydroxydopamine (6-OHDA) in destroying cardiac sympathetic terminals, we administered 6-OHDA (100mg/kg, 2 doses, 8-12 hours apart) in ovo by injecting through the airspace onto the inner shell membrane. [^{3}H]-NE uptake assays were performed 24, 48 and 72 hours after the initial injection of 6-OHDA. Uptake studies revealed that 6-OHDA was effective in reducing neuronal [^{3}H]-NE uptake as early as ID 12. Increases in uptake which occurred between 24-72 hours following 6-OHDA indicate ingrowth of new adrenergic fibers. To correlate cardiac catecholamine concentrations with innervation data, the concentrations (ng/mg tissue) of epinephrine (E) and norepinephrine (NE) in whole hearts were determined by high performance liquid chromatography. Beginning with ID 5, E levels rapidly increased to peak on ID 7 and then decreased and remained at low levels from ID 11 through ID 15. A smaller E peak occurred on ID 17 followed by a decrease which lasted through hatching. NE levels remained lower than E throughout the incubation period until just prior to hatching when NE surpassed E. At 2 weeks posthatching E was again greater than NE. We have observed that, generally, as adrenergic nerves arrive at the heart and innervation density increases (as determined by ^{3}H-NE uptake) catecholamine concentrations (primarily E) decrease accordingly. Between ID 17 and 19 there is a 2-fold increase in neuronal uptake and a 15-fold decrease in cardiac E concentration. It appears that circulating catecholamines may be an integral factor in adrenergic development and maturation. Supported by NIH Grant HD 17063.

52.2 IDENTIFICATION AND EXTIRPATION OF PRESUMPTIVE CARDIAC GANGLION CELL NEURAL CREST IN THE CHICK EMBRYO. M. L. Kirby and D. E. Stewart*. Dept. of Anatomy, Medical College of Georgia, Augusta GA 30912.

Presumptive cardiac ganglion cells can be identified in the bulbar plexus of the chick embryonic heart on the 4th day of incubation. These cells sprout processes and functionally innervate the heart by incubation day 12. The cells which become the cardiac ganglia presumably migrate from the neural crest which is the origin of all the autonomic ganglia. The cardiac ganglia are preganglionically innervated by the vagus nerve and should derive from the vagal area of neural crest which has been identified previously as the neural crest over somites 1-7. In the present study, we have identified the cardiac ganglion cell portion of the vagal neural crest using quail-chick chimeras and then examined the potential of a vagal cardiac ganglion cell lesion by removing various areas of the vagal cardiac neural crest. Using quail to chick chimeras, it was found that the vagal cardiac ganglion cells could be labelled reliably when the neural crest over somites 1 and 2 was transplanted. This type of transplant also resulted in labelling of pulmonary ganglia and the supporting cells (but not neurons) in the thoracic ganglia. Transplantation of neural crest above somite 1 resulted in labelling in cranial nerve ganglia. Transplantation of neural crest over somites 3-5 resulted in labelling of enteric ganglia with no labelling of either cardiac or pulmonary ganglia or supporting cells in the thoracic ganglia. The effectiveness of various neural crest lesions in producing a vagal aneural heart was evaluated using choline uptake in the atrium. Specific neuronal uptake of [^{3}H]-choline was defined as total uptake minus uptake in the presence of hemicholinium, a specific uptake blocker. Uptake in this system was saturable, temperature sensitive and Na^+ dependent. A normal study of [^{3}H]-choline uptake in developing chick atrium showed no choline uptake prior to 7 days of development. Uptake increased rapidly to peak at 10-12 days of development and then dropped to a plateau by 17 days. Bilateral and unilateral neural crest extirpations were performed at many different levels and several different stages of development. The embryos were allowed to develop to stage 34 (day 9) and the atria were assayed for [^{3}H]-choline uptake. [^{3}H]-choline uptake was lowest (40% of normal) following bilateral removal of neural crest over somites 1-3 at stage 9. Unilateral extirpation of this area of neural crest resulted in normal uptake at 9 days of incubation.
Supported by NIH Grant HD 17063

52.3 CATECHOLAMINE UPTAKE AND RELEASE BY CULTURED NEURAL CREST CELLS. G.D. Maxwell and P.D. Sietz*. Dept. of Anatomy, Univ. of Conn. Health Center, Farmington, CT 06032.

Embryonic neural crest cells in vivo give rise to the neurons of the autonomic and most sensory ganglia and, in addition, a variety of nonneuronal cell types. In tissue culture, some neural crest cells develop the capacity to synthesize and store the catecholamine norepinephrine (NE). It is of interest to learn what other neuronal functions, such as neurotransmitter release, are expressed by cells in these cultures.

Quail trunk neural crest cells were grown on thin collagen gels in medium containing 15% horse serum, 10% chick embryo extract and 5 ng/ml 2.5 S NGF. After 42 to 48 hrs. in culture, the neural tubes were carefully removed, leaving behind a population of cells that is highly enriched in neural crest cells. Neural crest cultures were incubated with 1.3μM [^{3}H]NE (specific activity 27 Ci/mmol) for 30 minutes. Cultures were washed and tested for their ability to release radioactivity in response to depolarizing conditions. Release was evoked in the presence of 40 mM K^+ with 1.3 mM Ca^{+2}. Radioactivity returned to baseline when cultures were returned to saline with 6 mM K^+ and 1.3mM Ca^{+2}. This release was inhibited (by > 90%) by the addition of 3 mM $CoCl_2$, indicating Ca^{+2} dependence of the release. The alkaloid veratridine (100 μM) also evoked a similar release of radioactivity from these cultures. Release in response to both conditions was seen after 7 days in vitro (the earliest time examined).

At the conclusion of release experiments, some cultures were processed for glyoxylic acid-induced fluorescence, diagnostic for catecholamines. The fluorescent cells generally had short processes (< 2 times the diameter of the cell body) with occasional longer processes seen on some cells. There was an excellent correlation ($r = 0.97$) between the number of fluorescent cells in a culture and the amount of evoked release. Other cultures were processed for autoradiography to locate radioactivity accumulated in cells. In these cultures, silver grains were observed above both processes and cell bodies in a subpopulation of cells in the culture.

These results indicate that neural crest cells grown in the absence of their normal synaptic inputs and targets develop the capacity to take up and release at least one neurotransmitter. This release is probably mediated by a subpopulation of catecholamine metabolizing cells in the culture.

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52.4 INCOMPLETE EXPRESSION OF A CATECHOLAMINERGIC PHENOTYPE IN CELLS THAT TRANSIENTLY APPEAR DURING DEVELOPMENT IN THE FETAL RAT GUT. M.D. Gershon, T.P. Rothman, G.N. Teitelman, T. Joh and D. Reis. Dept. Anatomy and Cell Biol., Columbia Univ. P&S, NY, NY 10032 and Dept. Neurology, Cornell Medical Coll., NY, NY 10021.

Proliferating cells that transiently express some catecholaminergic properties (TC cells) have been found in the gut and other organs of fetal rats and mice. These cells ultimately disappear from the bowel but it has not yet been determined whether their disappearance is due to a change in phenotype or elimination. When present, TC cells have been found to synthesize and contain catecholamine (CA). In rats, both tyrosine hydroxylase (TH) and dopamine beta hydroxylase (DBH) have been demonstrated in enteric TC cells by immunocytochemistry, while in mice the cells contain only TH. This suggests that TC cells may express only some of the constellation of properties that constitute the catecholaminergic phenotype of neurons. In order to test the hypothesis that catecholaminergic expression is incomplete in TC cells the ability of these cells to take up and store ^{3}H-norepinephrine was assessed directly. Fetal rat gut was removed on days E12 and E15 and incubated for 30 min with ^{3}H-NE in the presence of pargyline (0.1mM). Tissue was fixed and prepared for light and electron microscopic radioautography. Simultaneous immunocytochemical demonstration of TH served to mark TC cells. On day E12 no cells or processes were labeled radioautographically by ^{3}H-NE. TC cells, however, were abundant and well developed at this age. In contrast, the gut was heavily labeled by ^{3}H-NE on day E15, although TC cells could no longer be demonstrated with antibody to TH. TH-immunoreactivity was instead confined to axons and growth cones in the maturing enteric plexuses. These fibers also labeled with ^{3}H-NE and were probably the ingrowing axons of the sympathetic innervation of the gut. Although most of the labeling by ^{3}H-NE was of these axons, rare cells that did not display TH also took up ^{3}H-NE. At E12, therefore, TC cells contain TH but do not label with ^{3}H-NE, while at E15 another cell type is found that labels with ^{3}H-NE but does not contain TH. Cells that take up but do not synthesize CA have long been known to be constituents of the adult bowel and have been called amine handling cells (Furness and Costa, Cell Tiss. Res. 188:527-43, 1978). TC cells thus do not show all of the characteristics of the neuronal catecholaminergic phenotype. Uptake of NE following the disappearance of TC cells, therefore, does not indicate that these cells persist after they no longer express TH. The disappearance of TH-marked TC cells is followed by the innervation of the gut by sympathetic neurons and the appearance of amine handling cells. Supported by grants NS15547, BNS81-40896 and HL18974.

52.5 DEVELOPMENT OF SOMATOSTATIN AND VASOACTIVE INTESTINAL PEPTIDE IN THE RECTUM AND REMAK'S GANGLION OF THE CHICK. Miles L. Epstein° and June L. Dahl+, Departments of Anatomy° and Pharmacology+, University of Wisconsin Medical School, Madison, WI 53706

We have used immunocytochemistry to study the development of somatostatin (SOM)- and vasoactive intestinal peptide (VIP)-like immunoreactivity in the hindgut and Remak's ganglion of the chick. SOM-like immunoreactive cell bodies were first found in Remak's ganglion at 5 1/2 days of incubation (d.i.). Immunoreactive fibers were found at 7 1/2 - 8 1/2 d.i. in the wall of the rectum and were seen transversing the smooth muscle at 11 d.i. At 13 d.i., immunoreactive fibers were aligned parallel to the circular smooth muscle. In the newly-hatched chick, extensive SOM-like immunoreactivity was found in the myenteric and submucosal plexuses and in the circular smooth muscle. VIP-like immunoreactivity was first found in the wall of the rectum at 7 1/2 d.i. and cell bodies were clearly visualized in both the myenteric and submucosal plexuses at 9 d.i. VIP-immunoreactive fibers aligned parallel to the smooth muscle were found at 13 d.i. and were extensive by 17 d.i. Immunoreactive fibers were found in Remak's ganglion at that time but few or no cell bodies were visualized. We conclude that SOM cell bodies, which appear early in ontogeny in Remak's ganglion, provide extrinsic innervation to the hindgut of the chick. In contrast, VIP neurons appear to be intrinsic to the rectum and extend processes to Remak's ganglion.

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52.6 SHORT-TERM ACTIVATION AND LONG-TERM INDUCTION OF ADRENAL TYROSINE HYDROXYLASE DURING STRESS. S. J. Fluharty,* G. L. Snyder,* E. M. Stricker and M. J. Zigmond. Depts. of Biological Sciences and Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Recent studies from this laboratory indicated that after ivt 6-hydroxydopamine treatment hippocampal tyrosine hydroxylase (TH) is rapidly activated and this change persists until replaced by a long-term induction (Acheson & Zigmond, J. Neurosci. 1:493, 1981). The induction of adrenal TH has been shown to occur during prolonged stress. The present investigations determined whether this induction also is preceded by activation of the enzyme.

Male Sprague-Dawley rats were treated with phenoxybenzamine (PBZ, 20 mg/kg/day, ip), protamine zinc insulin (4-8 units/day, sc), or subjected to cold exposure (5° C) for varying times. Animals were then anesthetized with Equithesin and the adrenals removed and immediately frozen. Prior to the TH assay adrenal catecholamines (CAs) were removed by passing homogenates through a G-25 Sephadex column. This procedure lowered the K_m for pterin cofactor ($6MPH_4$) from 0.96 mM to 0.48 mM without altering V_{max}. TH was assayed by a coupled radioenzymatic decarboxylase microassay at a previously determined suboptimal pH (6.7) in the presence of saturating (10 mM) or subsaturating (0.1 mM) concentrations of $6MPH_4$.

Two days of PBZ treatment increased adrenal TH activity at both 0.1 mM and 10 mM $6MPH_4$ by 70-80%. This induction was apparently preceded by an activation; 30 min after the first PBZ injection, TH activity at 0.1 mM $6MPH_4$ was increased by 36% while at 10 mM $6MPH_4$ or at the pH optimum (6.2) it remained unchanged. Similarly, four days of insulin administration increased adrenal TH activity at both 0.1 and 10 mM $6MPH_4$ by 150-175%, and this induction was preceded by an activation of the enzyme (39% increase only at 0.1 mM $6MPH_4$, 30 min post-insulin). Finally, there was a clear induction of adrenal TH following 36-48 hrs of cold exposure (90-100% increase in the activity at both 0.1 and 10 mM $6MPH_4$). However, the enzyme was not activated 24 hr prior to the induction and experiments are now underway to determine whether an activation had occurred at some earlier time.

These results demonstrate the capacity of adrenal TH to respond rapidly to stress by increasing its activity and to maintain this increase when the stress is prolonged. These changes presumably help to couple CA synthesis and CA secretion during the increased demand associated with sympathetic nervous activity. It remains to be determined whether rapid activation of adrenal TH always occurs during stress, and when it does whether it persists until additional enzyme is induced.

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52.7 THE DEVELOPMENT OF TEMPERATURE-SENSITIVE NEURONS WITHIN THE PREOPTIC AREA AND ANTERIOR HYPOTHALAMUS OF DOMESTIC CHICK EMBRYOS AND HATCHLINGS. J.W. Reveley* and J.A. Boulant (SPON: B.T. Stokes). Department of Physiology, College of Medicine, Ohio State Univ., Columbus, Ohio 43210.

Previous studies have shown that certain neurons within the preoptic area and anterior hypothalamus (PO/AH) are sensitive to local hypothalamic temperature changes (T_H). Three types of units have been described within the PO/AH region: warm-sensitive, cold sensitive and temperature-insensitive neurons. It was the purpose of this study to examine the thermosensitivities of units within the PO/AH region during different stages of hypothalamic development.

Four stages of development were examined in the domestic chick *(Gallus domesticus)*: 15 days incubation, 19 days incubation, 4 days post-hatch and 12 days post-hatch. A thermode and thermocouple were stereotaxically placed in the rostral hypothalamus of urethanized birds. Hypothalamic temperature was held constant at 37°C, but could be rapidly changed between 32°C and 42°C to determine the thermosensitivity (m) of each unit.

In pre-hatched birds, 60% of the cells were classified as temperature-insensitive. Thirty percent of the cells were classified as either warm-sensitive (i.e. $m \geq 0.8$ impulses/sec/°C) or marginally warm-sensitive (i.e. $0.8 \geq m \geq 0.3$). Ten percent of the cells were classified as either cold-sensitive (i.e $m \leq -0.6$) or marginally cold sensitive (i.e. $-0.6 \leq m \leq -0.3$). The majority of warm-sensitive units were only sensitive to hyperthermic hypothalamic temperatures. In post-hatched birds, 50% of the cells were classified as temperature-insensitive. Forty percent of the cells were classified as warm or marginally warm-sensitive and 10% as cold or marginally cold sensitive. As the birds develop, there is a higher percentage of warm- and cold-sensitive cells vs. marginally warm- or cold-sensitive. In contrast to pre-hatched birds, there is a smaller percentage of warm-sensitive cells sensitive only in the hyperthermic range. Most of the warm-sensitive cells in the post-hatched birds were sensitive over wider ranges of hypothalamic temperature. In addition, there appears to be a time-course with respect to the developmental progression of neuronal thermosensitivity and spontaneous firing rate. This may be linked to the development of thermosensory afferents from skin and deep body thermoreceptors.

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53.1 NEUROTROPHIC MAINTENANCE OF MATURE SKELETAL MUSCLE: CHARACTERIZATION OF TROPHIC PRINCIPLE. H.L. Davis and J.A. Kiernan. Depts. of Clinical Neurological Sciences and Anatomy, The University of Western Ontario, London, Ontario, N6A 5C1, Canada.

Atrophy in a denervated muscle results from the disuse caused by paralysis of the muscle, and from the loss of special neurotrophic substances. Proteins extracted from rats' sciatic nerves have been shown to prevent the non-disuse atrophy of rats' extensor digitorum longus (EDL) muscles denervated for 7 days, when administered by daily intramuscular injections. The present investigation was undertaken to elicit more information about this neurotrophic phenomenon. Atrophy was assessed by measurement of wet weight, content of total protein and cross-sectional areas of types IIA and IIB fibres (in sections stained for ATPase) of EDL muscles.

Extracts of liver, which is a non-neural tissue, were injected daily into rats' EDL muscles denervated for 7 days. The rate of atrophy was as great as in controls, indicating that the trophic effects of the nerve extract were nerve-specific.

Normal muscles were injected with nerve extract for 7 days. They had wet weights and cross-sectional areas of IIA and IIB fibres identical to those of uninjected contralateral controls. This finding, in addition to those from earlier investigations, suggests that the amelioration of denervation atrophy by the nerve extract did not result from inflammatory processes.

Various doses of extract of rat or sheep sciatic nerves were injected into denervated rats' EDL muscles for 7 days. It was demonstrated that the action of the extract was dose-dependent but not species-specific. Greater concentrations of extract of ovine, than of rat, peripheral nerve were required for significant ameliorative effects.

Rats' EDL muscles were denervated for 14 days and were either not treated or were injected with nerve extract (optimal dosage) on all 14 days or only on the first or last 7 days. All denervated muscles injected with extract (for 7 or 14 days) exhibited significantly less atrophy than those that were not injected. Thus, injection of nerve extract caused reversal of non-disuse atrophy. The trophic effects of the extract persisted for at least one week.

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53.2 PURIFICATION OF A NEW NEUROTROPHIC FACTOR FROM MAMMALIAN BRAIN. Y.-A. Barde, D. Edgar* and H. Thoenen, Max-Planck-Institute for Psychiatry, Dept. of Neurochemistry, D-8033 Martinsried, FRG

Considerable evidence indicates that developing neurons depend on their environment for survival and differentiation. In order to understand the nature of these interactions, it is necessary to define the factors in the environment which are essential for the developing neurons in molecular terms. So far only one such factor has been purified and characterized - nerve growth factor (NGF). Isolation and characterization of other factors is particularly important in view of the fact that NGF has not yet been isolated from a source relevant to the physiology of the developing nervous system. The present report describes the isolation of a new neurotrophic factor from mammalian brain, the first to be purified since NGF. The assay system used to purify it was a quantitative determination of the number of surviving sensory neurons (isolated from chick embryos) cultured in the virtual absence of non-neuronal cells. The starting material was pig brain and the following purification steps were used: homogenization and acid treatment of the homogenate (pH 4.5), ammonium sulphate precipitation (70%), chromatography on CM-cellulose and hydroxylapatite, and preparative 2-dimensional gel electrophoresis. The material isolated by this procedure has the following characteristics: it is a very basic protein (pI ≅ 10.2) with a molecular weight of 12,300 which migrates as a single band on gel electrophoresis in the presence of sodium dodecyl sulphate. Approximately 1 µg can be isolated from 1 kg starting material after a purification of about 1.4 million-fold with a yield of 5%. The factor has a calculated specific activity of 0.4 ng/ml per unit (1 U permits 50% of maximal neuronal survival), which is essentially identical to that of NGF using the same assay system. This brain neurotrophic factor differs from NGF by immunological and functional criteria: antibodies to NGF do not block its activity and its effect on the survival of chick sensory neurons is additive to that of saturating concentrations of NGF. Furthermore, in contrast to NGF, the brain neurotrophic factor does not promote the survival of cultured chick or rat sympathetic neurons.

53.3 PURIFICATION AND CHARACTERIZATION OF NERVE GROWTH FACTOR FROM BOVINE SEMINAL PLASMA. G.P. Harper* and H. Thoenen. Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, 8033 Martinsried, West Germany.

Nerve Growth Factor (NGF) has been purified to homogeneity from bovine seminal plasma, which contains around 0.8 mg NGF/ml seminal plasma. NGF exists in the seminal plasma as a high molecular weight complex, analogous to the 7S form of NGF in the mouse submandibular glands. On acidification to pH 3, the bovine NGF complex dissociates into its subunits, revealing a highly basic (pI 9.5-10), low molecular weight (around 15,000 on SDS-polyacrylamide gel electrophoresis) protein with the NGF activity. This isolated bovine NGF thus appears to be rather more basic, and has a slightly longer peptide chain length, than the ß form of mouse NGF. As for mouse NGF, the native form of bovine NGF is a dimer of the peptide chains seen under denaturing conditions. Bovine NGF contains no carbohydrate moieties. The N-terminal amino-acid sequence of bovine NGF is very similar, but not identical, to that of mouse NGF; thus residues 3, 9 and 18, which are threonine, methionine and valine respectively in mouse NGF, are serine, arginine and isoleucine in bovine NGF.

Bovine NGF has exactly the same biological activities as mouse NGF in a wide variety of *in vitro* neural systems, which presumably indicates that the "active site" of NGF proteins (*i.e.* the part(s) of the molecule binding to the NGF-receptor in the cell membranes of target cells) is highly conserved. Such similarities are not seen, however, in the immunological properties of mouse and bovine NGFs, which differ substantially. For example, antisera against bovine NGF are only poorly effective in destroying the sympathetic nervous system (immunosympathectomy) of neonatal rats, and in inhibiting the biological activities of mouse NGF *in vitro*. The most likely explanation for these data is that the parts of the NGF molecule not directly involved in the active site are not subject to conservative evolutionary restraint, and in fact have diverged substantially.

53.4 TWO DISTINCT HIPPOCAMPAL GROWTH FACTORS IDENTIFIED IN VITRO. K.A. Crutcher and F. Collins*. Dept. of Anatomy, University of Utah School of Medicine, Salt Lake City, Utah 84132.

Sympathetic fibers invade the rat hippocampal formation or neocortex following cholinergic denervation of these brain regions. Previous studies have led to the suggestion that such sprouting could be due, in part, to the presence of a factor similar to nerve growth factor (NGF). In order to determine whether such a factor is normally present in the rat hippocampal formation (HF), extracts of this brain region were added to dispersed cell cultures of embryonic chicken ciliary (parasympathetic) or lumbar chain (sympathetic) ganglia. The neurons were plated onto culture dishes which were coated with a substratum-conditioning factor derived from heart-conditioned medium. The extent of neurite elongation from individual cells was measured two hours after exposure to medium alone or medium plus HF extract. The extract was prepared by homogenizing one HF in 2.5 ml of Hank's balanced salt solution and collecting the supernatant after centrifugation (100,000 g for 30 min.).

Exposure to HF extract accelerated the rate of neurite elongation from both parasympathetic and sympathetic neurons. As much as 100 fold dilution of the HF extract still resulted in significant acceleration of neurite elongation. NGF antiserum was added in order to determine whether the HF extract activity could be antagonized. The response of sympathetic neurons was reduced to control levels by the NGF antiserum but the response of parasympathetic neurons was unaffected. NGF antiserum alone had no effect on neurite elongation.

These results provide evidence for the presence of at least two distinct growth factors within the rat HF, one of which may be related to NGF. The sprouting of sympathetic fibers following septal lesions and the affinity of septohippocampal axons for exogenous NGF suggest that a similar factor might be responsible for the normal development of central cholinergic pathways and for sympathetic sprouting following cholinergic denervation. The presence of discrete growth factors might provide the basis for the specificity of other examples of neuronal sprouting within the mammalian CNS.

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53.5 SKELETAL MUSCLE CELLS PRODUCE A FACTOR WHICH PROMOTES NEURITE GROWTH IN CULTURE. Raphael Gruener and Judy Christiansen*. Department of Physiology, University of Arizona College of Medicine, Tucson, AZ 85724, U.S.A.

Trophic control by target tissues of their innervation has been well-documented, for the sympathetic nervous system, to be mediated by target-elaborated nerve growth factor (NGF). It is becoming apparent that this upstream regulation may be a general phenomenon. We have therefore examined the possibility that embryonic skeletal muscle cells produce a factor which influences neuronal maintenance, promotes neurite production and provides them with directionality.

Spinal cord cells, from Xenopus laevis embryos, were grown in the presence of defined muscle-conditioned medium (MCM) free of serum and embryo extract. MCM was obtained from embryonic Xenopus muscle cell cultures after 5 days incubation and used as the growth medium for nerve cell cultures. The number of neurites, produced in the culture, as well as the number of neurite-producing neurons, was examined. The effects of MCM were also examined in cultures of PC12 cells and in superior cervical ganglia explants from fetal rat and compared to the effects of NGF. Conversely, the effects of NGF were assayed on Xenopus nerve cell cultures.

We have found that neurite production is greatly enhanced by MCM. These effects are similar to those found for NGF with both factors, however, exerting only target-specific activity. We have also found that the MCM is heat inactivated and is not dialyzable. Finally, pretreating culture chambers with MCM is ineffective in reproducing MCM effects on neurite growth.

We conclude that MCM contains a muscle-elaborated, possibly specific, factor which may be important developmentally and in the adult maintenance of upstream trophic regulation.

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53.6 CYCLIC AMP MAY MEDIATE THE EFFECT OF NGF ON PHOSPHOLIPID METABOLISM, A.E. Traynor The Salk Institute, P.O. Box 85800, San Diego, CA 92037

The NGF induced differentiation in PC12 cells may be mediated by cyclic AMP and calcium (Schubert, D. et. al., Nature, 273:718, 1978). Since NGF increases phosphatidyl-inositol (PI) turnover in these cells (Traynor and Schubert, in preparation) we studied the effects of cyclic AMP and calcium ionophore (A23187) on the PI response. Cyclic AMP (10^{-3}M) was half as effective as NGF in stimulationg PI labelling with $^{32}PO_4$. A23187 stimulated PI labelling in the presence but not the absence of calcium. These results were consistent with the interpretation that PI turnover is preceeded by increased cyclic AMP and calcium flux. However, depolarizing the cells with KCl increased calcium flux and resulted in the initiation of neurite extension but did not stimulate PI labelling. Therefore neurite extension does not require increased PI turnover.

53.7 RETROGRADE TRANSPORT OF NERVE GROWTH FACTOR (NGF) IN GOLDFISH RETINAL GANGLION CELLS. H. K. Yip* and E. M. Johnson (SPON: V. S. Yip). Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Previous studies have shown that NGF, administered at the time of crush, but not 24 hours after, enhances regeneration of goldfish optic nerve. NGF was effective when administered either by intraocular injection or by local application to the lesion site. The site and mechanism of action of NGF is not understood. One of the possibilities is that NGF is retrogradely transported, by either a receptor mediated or non-receptor mediated process, to the cell body and exerts its growth-promoting effect.

For the present study, attempts were made to demonstrate the retrograde transport of ^{125}I-NGF in the normal animal by injection in the optic tectum. The amount of radioactivity transported to the ipsilateral and contralateral retinas was measured in a Gamma counter. Retinas were also processed for autoradiography. No retrograde transport was observed in these animals, suggesting that the retinal ganglion cell processes do not have NGF receptors.

In contrast, when ^{125}I-NGF was microinjected at the optic nerve crush site at the time of lesion, retrograde transport to the ipsilateral retina, but not contralateral retina, was observed. Radioactivity was detected in the ipsilateral retina 2 hours after the injection, peaked at 6 hours and returned to base-line by 16 hours. Autoradiographic examination showed that about 10% of retinal ganglion cells were labelled. No labelling was seen in other retina cells. Injection of ^{125}I-NGF at the site of lesion, 16 or 24 hours after the crush, produced no transport to the ipsilateral retina. These data, therefore, suggest that goldfish retinal ganglion cells do not have NGF receptors and do not normally transport NGF. However, there is a temporal correlation with the ability of NGF to be transported after a lesion to the retinal ganglion cells and its ability to enhance regeneration after a lesion. We suggest that NGF may be capable of exerting positive effects on cells which do not have surface receptors for the factor.

53.8 PRIMARY AMINE SECRETION FROM NEUROMUSCULAR PREPARATIONS. J.R. Musick. Dept. Physiology, Sch. of Med., U. of Utah, Salt Lake City, UT 84108.

Recent investigations using cultured cells to assay for putative trophic factors indicate the motor neurons contain substances affecting skeletal muscle properties and that muscle contains substances affecting motor neurons. Active factors appear to be proteinaceous, including macromolecules and a small peptide(s). Some long-term neuromuscular interactions may be mediated by secretion of trophic factors. In the present study, the fluorescamine assay was used to study the spontaneous secretion of primary amines from the R. catesbeiana sciatic nerve-sartorius muscle. The excised preparation was superfused at 0.35 ml/min with physiological saline and effluents were collected in 10-min intervals for up to 7 hrs. The primary amine efflux decayed according to a double exponential (τ_1 = 6.90 ± 1.0 hrs; τ_2 = 47.6 ± 7.9 hrs; n = 10) following the maximum efflux rate ($\bar{\chi}$ = 2.99 pmoles Leu eq/min/mg). Pooled and concentrated effluents were separated into four primary amine-containing fractions (I to IV) of apparent molecular weight ≥ 1800, 425, 150 and 50 daltons, respectively, by chromatography on Bio Gel P-2.

Fraction III, which contained 78% of the recovered amines was subject to more detailed analysis. It contained the following free amino acids: Lys, His, Arg, Thr, Ser, Glu, Gly, Ala, Met, Ile and Leu. Acid hydrolysis of an equamolar sample increased the amount of 14 amino acids suggesting that fraction III contains a peptide(s) with the following composition: (Lys, Arg, Asx, Thr, Ser_2, Glx_4, Pro, Gly_5, Ala_3, Met, Ile, Leu_2, Tyr, Phe). Further experiments were performed to determine the number of peptides in fraction III. Primary amines and UV absorbing material (λ_{max} 275-280 nm) eluted in the void volume and were separated from smaller amines when fraction III was run on a Sephadex G-10 column using a mixture of phenol, acetic acid and water as an eluent. These results suggest that fraction III contains an aromatic peptide(s) that is ≥ 700 daltons. Fluorescamine derivitives of fraction III were analyzed by reverse-phase HPLC and the initial observations indicated the presence of 2 peptides.

It is concluded that in addition to the well-known secretion of proteins and amino acids, peptides are also spontaneously secreted from neuromuscular preparations. Chemical assay of primary amine secretion at the resolution levels afforded by analytical HPLC columns allows investigation of the parameters affecting secretion of specific molecules. In addition, HPLC purification of secreted peptides allows determination of their chemical structure as well as biological activity.

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53.9 NERVE GROWTH FACTOR SYNTHESIZED BY MOUSE FIBROBLAST CELLS IN CULTURE: ABSENCE OF α AND γ SUBUNITS. N. J. Pantazis. Dept. of Anatomy, Univ. of Iowa, Iowa City, IA 52242.

Two high molecular weight forms of Nerve Growth Factor (NGF) have been reported in the mouse submandibular gland, 7S-NGF (Varon, S., Nomura, J. and Shooter, E.M., Biochemistry, 6:2202, 1967) and NGF_1 (Young, M., Saide, J.D., Murphy, R.A. and Blanchard, M.H., Biochemistry, 17:1490, 1978). Both of these forms apparently contain subunits named α, γ and β-NGF. Although mouse salivary glands contain high concentrations of NGF, the salivary glands of other animals contain very little. Therefore, the source of NGF in most animals has not been identified. It is possible that low amounts of NGF are synthesized by many tissues in the animal to locally maintain the peripheral nervous system. The fact that various cell types in vitro synthesize and secrete NGF into their feeding medium (conditioned medium) supports this hypothesis.

What are the biochemical properties of NGF synthesized outside of the mouse salivary gland? Previous work using mouse L929 fibroblast cells in culture (Pantazis, N.J., Blanchard, M.H., Arnason, B.G.W. and Young, M., Proc. Natl. Acad. Sci. USA, 74: 1492, 1977) determined that a protein very similar to β-NGF was synthesized by these cells. Recently, radioimmunoassays have been established which are specific for the α and γ subunits of 7S-NGF. These assays could not detect either α or γ in concentrated samples of fibroblast conditioned medium, whereas the concentration of β-NGF in these samples was 700-800 ng/ml. When samples of pure 7S-NGF from mouse submandibular gland were assayed, the α and γ subunits were readily detectable (β-NGF = 34 μg/ml; α = 57 μg/ml; γ = 94 μg/ml). Since apparently no α or γ is synthesized by fibroblast cells, it would appear that the 7S-NGF molecule is not produced by these cells.

Gel filtration studies of the conditioned medium indicated that a molecule containing β-NGF but not α or γ was present. The molecular weight (M_r) of this complex was greater than 300,000 which is much larger than either 7S-NGF (140,000 M_r) or NGF_1 (116,000 M_r). Mouse fibroblasts, therefore, appear to make a previously undescribed form of NGF which is different from the salivary gland forms of NGF in two respects. The fibroblast molecule has a larger molecular weight and it lacks α and γ subunits. The properties of this new form of NGF are under investigation.

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53.10 SPINAL CORD NEURONOTROPHIC FACTORS. W. Luyten*, M. Manthorpe, and S. Varon. Depts. of Neuroscience and Biology, Sch. of Med., Univ. of Calif. San Diego, La Jolla, CA 92093.

Various conditioned media (CM) are capable of supporting the 24 hr in vitro survival of dissociated 4-day (Stage 23) chick embryo lumbar spinal cord neurons. Tritration of CM from cultures of chick embryo heart and skeletal muscle cells, rat RN22 Schwannoma, C-6 glioma, astroglia and oligodendroglia and the chick embryo lumbar cord cells themselves revealed trophic activity; at high concentrations most CM also show a neuronotoxic activity. The molecular properties of all these CM-derived factors were similar and somewhat unexpected being heat (90°) and trypsin-resistant and smaller than MW 10^4. The effects of the Schwannoma (RCM) and lumbar cord (LCM) media on neuronal survival and choline acetyltransferase (CAT) activity were further examined in high (3×10^4 cells/cm^2) and low (3×10^3 cells/cm^2) cell seeding density cord cultures.

Lumbar cord cells were seeded on polyornithine-bound neurite promoting factor (J. Neurochem. 37: 759, 1981) in serum free N1 medium (Exp. Cell Res. 125: 183, 1980) as described previously (Dev. Brain Res. 3: 277, 1982). The proportion (%) of seeded cells which survived as neurons was counted at 1 and 5 d using phase-contrast microscopy, and replicate cultures harvested for subsequent CAT assay. The following results were obtained:

1. At low density few (approximately 10%) neurons survive 1 d and none for 5 d.
2. The presence of RCM in low density lumbar cultures dramatically increased survival to 70% by 1 d but none survived for 5d.
3. At high density a considerable neuronal survival (40%) was evident at 1 d and by 5 d 15% were still alive. This density-dependent neuronal survival is shown to be due to a soluble trophic factor present in the high density cultures (but not detectable in low density ones) which is capable of supporting neuronal survival for 1 and 5 d in low density cultures.
4. RCM does not increase 1 d neuronal survival significantly in high density cultures but decreases the expected 15% at 5 d to less than 1%.
5. CAT activity measurements reveal the presence of motoneurons in all surviving populations. As in vivo, there is a temporal increase in CAT activity in culture.

The observation of toxic influences in material which also contains survival-promoting factors deserves further study considering its potential relevance to neuropathology and normal development.

53.11 IONIC CONTROL BY NERVE GROWTH FACTOR IN MONOLAYER NEURONAL CULTURES. Stephen D. Skaper and Silvio Varon. Dept. Biol., Sch. of Med., Univ. of Calif. San Diego, La Jolla, CA 92093.

Nerve Growth Factor (NGF) is required for the survival and development of certain sensory and sympathetic neurons. We have demonstrated previously that, in the absence of NGF and before irreversible changes take place, NGF-responsive sensory and sympathetic ganglionic cells in suspension lose their ability to maintain normal intracellular levels of Na and K. Addition of NGF to such factor-deprived cells results in a restoration of intracellular Na and K within minutes and in a coupled fashion. These and other data have shown that NGF controls the performance of the Na, K-pump in its target ganglionic neurons.

In the present study we have examined the Na and K behaviors, under the influence of NGF, of monolayer cultures from embryonic day 8 (E8) chick dorsal root ganglionic (DRG) neurons. An enriched (75-80%) neuronal preparation obtained by means of a selective attachment step over plastic is seeded into 16 mm culture wells containing a polyornithine substratum, and Eagle's Basal Medium supplemented with fetal calf serum and NGF. After 18 hr the medium is replaced with fresh medium containing NGF or no NGF. ^{86}Rb (as a tracer for K) is added at this time. Over the next 12-15 hr the cultures are assessed for numbers of surviving neurons and radioactivity accumulated by the cells. Cultured E8 chick DRG neurons become unable to maintain their intracellular K level when deprived of exogenous NGF over 4-6 hr. The NGF-deprived and K-depleted neurons reaccumulated K within minutes upon delayed NGF presentation. The occurrence of this K response in culture to exogenous NGF parallels the response seen for enriched E8 neuronal suspensions, including the time at which irreversibility of the K deficit first becomes detectable. Similar experiments performed with ^{22}Na indicate corresponding ionic behaviors for cultured E8 DRG neurons. These consequences of the presence or absence of NGF are observed at E7 and E10 but not at E14, paralleling a previously observed survival response to NGF of E7 and E10, but not E15 chick DRG neurons in culture. This monolayer culture system should provide numerous opportunities for examining the consequences on neuronal survival of the ionic effects of NGF.

53.12 β-NERVE GROWTH FACTOR-LIKE PROTEINS IN HUMAN FETAL SMALL INTESTINE AND ADULT MOUSE BRAIN. M.B. Rosenberg[1*], M.H. Grossman[1], E. Hawrot[2], J.E. Pintar[1*], J.P. Schwartz[3], and X.O. Breakefield[1]. Depts. [1]Human Genetics and [2]Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510, and [3]NIMH, Washington DC 20032.

In rodents, β-nerve growth factor (β-NGF) is essential for the development and maintenance of sympathetic and sensory neurons. This protein has been purified from mouse submaxillary glands, where it is present in large concentrations, and well characterized, but little is known about β-NGF in other mouse tissues or in human tissues.

We have detected proteins in human fetal small intestine and adult mouse brain extracts that resemble mouse submaxillary β-NGF in receptor binding ability and in antigenic determinants. Extracts were tested for competition with ^{125}I-labeled mouse submaxillary β-NGF for binding to receptors on rat pheochromocytoma (PC-12) cells. β-NGF-like proteins were present in small intestine and mouse brain extracts at concentrations of 3-5 ng per mg total protein.

Small intestine extracts were also tested for antigenic resemblance to mouse submaxillary β-NGF. Proteins were separated by SDS polyacrylamide gel electrophoresis, transferred electrophoretically to nitrocellulose sheets, and screened with rabbit antisera to purified mouse β-NGF (donated by E. Johnson, G. Guroff and L. Greene), or with non-immune serum. Two immunospecific bands of molecular weight 24,000 and 43,000 were detected with 2 of 7 antisera tested. A two-site radioimmunoassay, using commercially prepared affinity purified antibodies to mouse submaxillary β-NGF, detected no β-NGF-like determinants in small intestine extracts. Small intestine extracts showed no biological activity using a bioassay involving induction of neurite outgrowth from chick dorsal root ganglia.

These studies indicate that human fetal small intestine contains some protein(s) that binds to β-NGF receptors on PC-12 cells but does not elicit a biological response from chick dorsal root ganglia neurons, and that shares some antigenic determinants with mouse β-NGF. In addition, a β-NGF-like protein that binds to β-NGF receptors on PC-12 cells appears to be present at low levels in adult mouse brain. Studies are underway to determine whether the mouse brain β-NGF-like protein is biologically active and structurally related to β-NGF from the mouse submaxillary gland. J. E. Pintar present address: Division of Endicrinology, Mt. Sinai Sch. Med., NYC, NY

53.13 IN SITU LOCALIZATION OF NGF RECEPTORS IN NEONATAL MICE. P. Bernd and L.A. Greene*. Department of Pharmacology, New York University Medical Center, New York, NY 10016.

Nerve Growth Factor (NGF) has been shown to be critical in the normal development of sympathetic and some sensory neurons. It is not known, however, when these receptors for NGF appear and/or disappear. It is also possible, that other targets for NGF may exist during development. A method of whole body radioautography has been devised in order to localize at the microscopic level possible targets for NGF throughout the body of a neonatal mouse. Animals (postnatal day 1) were injected subcutaneously with iodinated NGF (^{125}I-NGF; 3.88 x 10^4 CPM/ng; 30 ng/g body weight), perfused intracardially with saline and then fixative after 1 to 2 hours (3% glutaraldehyde in 0.1 M potassium phosphate buffer, overnight), dehydrated, embedded in paraffin, sectioned (10 µm), deparaffinized, exposed to Ilford L4 emulsion for 1 to 8 weeks, developed, fixed and stained with hematoxylin and eosin. In addition, certain organs of interest were embedded in Epok 812 and examined by electron microscopic radioautography (flat substrate technique). Control animals were injected with a 100-fold excess of nonradioactive NGF (3 µg/g body weight) in addition to the ^{125}I-NGF. In this manner, specific in situ NGF receptors can be identified.

Examination of 10 µm sections revealed that labelling was localized to previously described targets for NGF, such as dorsal root ganglia and superior cervical ganglia. Labelling was also detected over peripheral nerves, as well as the adrenal medulla. It was also interesting to note that a stream of labelled cells were seen migrating into the adrenal medulla from a nearly paravertebral ganglion that was also labelled. The adrenal medullary cells, therefore, appear to have receptors for NGF prior to reaching their final destination and becoming fully differentiated. The thyroid gland was also found to accumulate label, but electron microscopic radioautography revealed the grains to be localized over the follicular colloid, probably resulting from the uptake of free ^{125}I. Label did not appear to be specifically localized in other peripheral tissues or in the central nervous system. In control experiments, none of the above structures were found to be labelled. It is anticipated that this technique will be applicable to the localization of NGF receptors in prenatal animals. This study was supported by NIH grant #NS 16036, March of Dimes grant #1-704 and a Pharmaceutical Manufacturers Association Foundation fellowship (PB).

53.14 NERVE GROWTH FACTOR (NGF)-DEPENDENT PHOSPHORYLATION IN PHEOCHROMOCYTOMA CELLS. P.J. Seeley* and L.A. Greene* (SPON: A. Chalazonitis) Dept. Pharmacology, NYU Medical Center, New York, NY 10016.

The clonal rat pheochromocytoma line PC12 responds to NGF by cessation of cell division and assumption of the phenotype of a sympathetic neuron. We have examined the influence of NGF on phosphorylation patterns of PC12 and cloned variants of this line. Cultures were labeled with [^{32}P]-inorganic phosphate by incubation at 37°C in a HEPES-buffered Krebs solution. Cell components were fractionated by SDS-polyacrylamide gel electrophoresis and the gels processed for autoradiography. Exposure of PC12 cells to NGF for 1h caused increased phosphorylation of bands corresponding to MWs of ca. 54, 59 and 64kD and decreased phosphorylation of a 62kD band. A significant proportion of the 59kD band represents tyrosine hydroxylase (K. Lee et al., these abstracts). Treatment with 1mM db-cAMP plus or minus 200µM IBMX or with 60mM KCl or 5µM A23187 for 1h enhanced the 59kD band, but to a lesser extent than treatment with NGF. Db-cAMP did not mimic NGF-induced changes in the 62 and 64kD bands, though it did enhance phosphorylations which were not altered by NGF alone (e.g. 14kD). These data are not consistent with a simple cAMP messenger system for NGF. A set of cell responses to NGF which partially overlapped with the above set was detected by alkaline hydrolysis of gels prior to autoradiography. In this case there were enhancements of 40, 46, 59 and 64kD bands.

PC12 cells which had been treated with NGF for $\geq$7d responded to 1h readdition of NGF (after 4h of withdrawal) with increased phosphorylation of 54 and 59kD bands. The 59kD response was markedly less than that for cells without NGF pretreatment. Sister cultures withdrawn from NGF for 4h and then treated with 1mM db-cAMP for 1h had enhanced phosphorylation not only in the 54 and 59kD bands but also at 14 and 57kD. Similar additions of 60mM KCl or 5µM A23187 for 1h gave some enhancement of the 59kD band. There was also, on long-term treatment with NGF, a strong increase in a band at $\geq$300kD which appears to be the microtubule associated protein MAP1.

Cloned variants of PC12, which have restricted responsiveness to NGF, are altered in their phosphorylation pattern on 1h exposure to factor compared with the parent line. In particular, enhancement of the 59kD phosphorylation by NGF is less for variant cells. Basal phosphorylation profiles are different and there are additional NGF-responsive bands for the variant series.

Study of phosphorylation in these systems is a prospectively incisive means of examining second messenger signalling for NGF. This work was supported by grants NS 16036, NS 17888 and from the March of Dimes.

53.15 CALCIUM ENHANCES THE EFFECT OF BRAIN EXTRACT ON ACETYLCHOLINE RECEPTOR LEVELS IN CULTURED RAT MYOTUBES. J. R. Bostwick,* R. B. Moore* and S. H. Appel. (SPON: A. Coats). Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

The number of surface acetylcholine receptors (AChR) in cultured rat myotubes increased 60% when post-fused cells were incubated for four days in medium supplemented with a soluble extract of fetal calf brain. This effect was enhanced by increasing the calcium concentration from 2 mM to 15 mM where a three-fold increase in AChR was observed. Magnesium did not substitute for calcium. In 15 mM Ca^{2+} medium the effect of extract was dose-dependent and saturable. Pre-incubation of the brain extract with trypsin (0.1%, 37°C, 90 minutes) rendered it inactive whereas heating the extract in a boiling water bath for 10 minutes did not reduce the stimulatory effect. Extract-treated myotubes accumulated surface AChR at rates up to 4.3 times higher than controls when measured over 12-hour intervals during the third and fourth days of incubation. In contrast, overall protein synthesis as measured by incorporation of ^{14}C-amino acids was not increased. A proteinaceous aggregate was formed when brain extract was incubated in 15 mM Ca^{2+} medium. This aggregate, but not the remaining medium supernatant, produced the increase in AChR levels when added to cultured myotubes. Activity remained when the aggregate was boiled or redissolved in 20 mM EGTA but not when pre-treated with trypsin. An SDS-PAGE analysis of the aggregate revealed three major protein bands, two of which were not predominant in the protein profile of the brain extract. Furthermore, these two protein bands were not present in an inactive aggregate formed from the extract in serum-free 15 mM Ca^{2+} balanced salt solution. This indicates that the active aggregate may constitute a complex derived from both brain extract and serum proteins. Increases in AChR number were not caused by non-specific effects of the aggregate on myotubes since an aggregate formed from liver extract did not elicit the response. Fractionation of the calf brain extract by high performance liquid chromatography produced 5 discrete peaks of protein. Material eluted at the exclusion limits of the column (> 200,000 daltons) formed an aggregate when added to 15 mM Ca^{2+} medium and increased AChR levels on myotubes. Analysis of this fraction on PAGE showed a single band with a molecular weight of 55,000. This band is predominant in the active aggregate and the whole extract. (Supported in part by grants from the John A. Hartford Foundation and the Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation.)

53.16 SEPARATION OF TWO FACTORS FROM SKELETAL MUSCLE WHICH SUPPORT CILIARY NEURON SURVIVAL AND DIFFERENTIATION. Ken Vaca*, James McManaman*, and Stanley H. Appel. Dept. of Neurology, Program in Neurosciences, Baylor College of Medicine, Houston, Texas, 77030.

Chick ciliary ganglion neurons in dissociated cell culture provide a relatively homogeneous, convenient and sensitive system with which to test for macromolecules which support the survival, growth and differentiation of motor neurons. When grown in a defined medium (Bottenstein & Sato, PNAS 76: 514-517) on a poly-lysine substrate, the cells become pyknotic after 3 to 5 days in culture and degenerate and detach shortly thereafter unless appropriate supplemental macromolecules are provided. In the chick embryo, striated muscle was found to be a particularly good source of such trophic factors which promote the survival and morphological and biochemical differentiation of these motor neurons. Soluble extracts, dialyzed (M.S. cutoff 12-14,000) after high speed centrifugation, of skeletal muscle from several different species were similarly effective. Trophic activity was further purified, using fetal calf muscle as a source, by a series of salt fractionation and column chromatography steps. Two fractions which supported the long-term ($\geq$ 2weeks) survival and differentiation of the ciliary neurons were obtained. One fraction (A) caused the neurons to interconnect in a lush network and the proliferation of non-neuronal cells which often aligned with one or more neuritic processes. Fraction A also enhanced the ability of the cells to acetylate exogenously provided choline. Another fraction (B) failed to support any non-neuronal cells for more than a week in culture but caused a dramatic extension of long, narrow neurites. Furthermore, fraction B was more effective than A in increasing neurotransmitter synthesis, even though survival was somewhat less per culture. Fraction B was purified approximately 1000-fold from the soluble muscle extract. A high K^+ (30 mM) medium increased ACh synthesis to a similar extent, as did partial depolarization with 5 µM veratridine, while having far lesser effect on morphology. The CA^{++} antagonist D600 (10 µM) totally blocked the effect of high K^+ but not fractions A or B. Thus, skeletal muscle may contain more than one distinct macromolecule involved in the control of motor neuron growth and maintenance. These may act independently or in concert with electrical activity to modulate neural development.
(Supported in part by grants from the John A. Hartford Foundation and the Robert J. Kleberg and Helen C. Kleberg Foundation)

53.17 HEART CONDITIONED MEDIUM ELICITS POSTLESION MUSCARINIC RECEPTOR RECOVERY IN VIVO. Amy Rothman Schonfeld, Leon Thal*, Sara G. Horowitz* and Robert Katzman. Dept. of Neurology, Albert Einstein College of Medicine, Bronx, NY. 10461.

Heart Conditioned Medium (HCM) contains a neurotrophic factor which supports the survival and neuritic outgrowth of dissociated peripheral cholinergic neurons in vitro (Helfand et. al., Devel. Bio. 50:541, 1976). Recent work in our laboratory demonstrated that intraseptal administration of HCM stimulates the regenerative growth of injured central cholinergic fibers into iris implants placed in the hippocampus of rats in vivo (Schonfeld et. al., Brain Res. 229:541, 1981; Schonfeld and Katzman, Soc. Neurosci. (abst.) 7:677, 1981). The aims of the present study were to monitor muscarinic cholinergic receptor concentrations during denervation and central innervation of the peripheral tissue targets and to evaluate the effect of HCM on these changes.

For this, heterologous iris implants were inserted in the anterodorsal hippocampus in female rats previously subjected to ipsilateral superior cervical ganglionectomies. At the same time, a cannula was stereotaxically placed in the medial septum. Animals were administered 1 µl of either HCM (prepared by Dr. R. Johnston of Stanford U. according to Helfand et. al. (1976)) or saline as control vehicle. The injection schedule varied with the length of treatment in order to maintain the extent of injection-induced trauma relatively constant among the groups: 0, 4 and 8 days: daily; 16 days: alternate days and 28 days: 3 per week. After decapitation, the brains were rapidly removed, irides were dissected free from surrounding brain parenchyma, weighed and frozen. Levels of cholineacetyltransferase (CAT), the marker enzyme for cholinergic regeneration (Emson et. al., Brain Res. 135:87, 1977), were measured according to Datta, Thal and Wajda (Brit. J. Pharm. 41:84, 1971). Concentrations of muscarinic cholinergic receptors in implants were determined using (^{3}H)-quinuclidenyl benzilate (QNB) as the ligand (Yamamura and Snyder, Proc. Natl. Acad. Sci. 71:1725, 1974).

The results indicate that: (1) a dramatic drop in the number of muscarinic receptors is observed 4 days after denervation; (2) under control conditions, central cholinergic innervation of implants is not associated with muscarinic receptor recovery; and (3) after HCM administration, muscarinic receptor levels begin to increase within 2 weeks and approach the prelesion endogenous concentration following 28 days of treatment. These results support the hypothesis that trophic factors may facilitate the restoration of effective, appropriate connections between nerve fibers and their targets.

53.18 EXTRACT FROM BRAIN STIMULATES NEURITE OUTGROWTH FROM FETAL RAT RETINAL EXPLANTS, J. E. Turner, Y. A. Barde, M. Schwab and H. Thoenen. Department of Neurochemistry, Max-Planck Institute for Psychiatry, 8033 Martinsried, F-R, Germany and Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

The conditioned media from glioma cells (GCM) has been shown to possess neurotrophic qualities when tested with dissociated embryonic peripheral ganglionic neurons (Barde, Y. A., et al., Nature, 274: 818, 1978). In addition, a crude rat brain extract has been shown to enhance the survival of embryonic peripheral neurons in a manner similar to the GCM and was shown not to be NGF. This and other evidence points very strongly to the fact that there are neurotrophic factors in the CNS and that these factors may be elaborated by glial cells. However, these putative factors have not been tested in the developing mammalian CNS. We will report for the first time that a fraction from a pig brain extract (BE) stimulates neurite outgrowth from fetal rat retinal explants.

When BE was added in increasing concentrations to the culture medium, there was a dose dependent increase in the density and length of neurite outgrowth from the explants between 1-100 µg/ml, leveling off between 100-500 µg/ml. The BE mediated increase was approximately 5-fold at 100 µg/ml when compared to control values. BE administration (50 µg/ml) to retinal explants caused a continued, statistically significant, elevation of growth over controls for at least two weeks in culture. Control explants ceased neurite outgrowth after day 11, and subsequently began to show signs of degeneration. In order for BE to elicit its effects it must be present in the medium: BE will not bind to the polyornithine substrate, since no stimulatory effects were noted when explants were placed in control medium after the substrate had been pretreated with BE. Exposure of the explants to NGF or NGF-As had no effect on neurite density or length compared to controls. In addition, NGF did not augment the BE mediated response when added together nor did the combination of BE + NGF-As significantly reduce the NGI's compared to BE alone.

It is suggested that such an extract can be used as a source of putative neurotrophic factors exhibiting in the mammalian CNS an action similar to that of NGF in the PNS of mammals and in the CNS of lower vertebrates like fishes and amphibia.

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53.19 IMMUNOHISTOCHEMICAL LOCALIZATION OF NEURITE EXTENSION FACTOR IN ADULT RAT BRAIN. D. Kligman* and D. M. Jacobowitz (SPON: G.H. Burrows). Lab. of Biochem. Genetics, NHLBI, NIH and Lab. of Clin. Science, NIMH, Bethesda, MD 20205.

Neurite extension factor (NEF) is an acidic protein (Mr = 75,000) isolated from bovine brain which induces neurite outgrowth from chick embryo cerebral cortex neurons in vitro in defined medium (Kligman, D., Brain Research, in press). A monospecific antiserum against NEF (anti-NEF) has been prepared in rabbits by repeated intradermal injections of the purified protein. Immunoperoxidase staining of nitrocellulose blots of total soluble bovine brain proteins separated by SDS polyacrylamide gel electrophoresis under non-reducing conditions reveals only a single stained band (Mr = 75,000). Additionally, anti-NEF IgG bound to protein A-Sepharose quantitatively adsorbs NEF bioactivity from soluble bovine brain extract.

Immunofluorescence microscopy has been used to localize NEF in adult rat brain. Brains were removed from formalin-perfused rats. Cryostat sections were treated with anti-NEF (1:1,000 dilution in PBS) for 1-5 days, followed by fluorescein-conjugated goat anti-rabbit IgG. NEF appears to be localized in the cytoplasm of neurons, not glial cells. In normal rat brain, fluorescent neurons (both cell bodies and fibers) were observed in the cerebral cortex. Fluorescent fibers were visible in the cerebellum, some of the cranial nerve tracts, the olfactory bulb (outer nerve fiber layer) and the reticular formation. The optic nerves were negative. Animals treated with colchicine intraventricularly (75 µg/25 µl PBS), perfused 2 days later, and stained with anti-NEF revealed additional fluorescent neurons; cell bodies were noted in the hippocampus, thalamus, hypothalamus, and amygdala, while axonal processes were noted in the hippocampus. Intraventricular colchicine appears to cause an endogenous buildup of NEF in discrete brain regions which are not immunohistochemically positive for NEF in normal brain. Localization of NEF (a molecule that is biologically active on embryonic neurons) in distinct regions of adult rat brain, some of which are known to sprout after lesioning (e.g. the outer nerve fiber layer of the olfactory bulb or the hippocampus), suggests a possible role for this molecule in neural plasticity and regeneration.

(Supported in part by the Muscular Dystrophy Association.)

53.20 PERIPHERAL NERVE EXTRACT: DEVELOPMENTAL APPEARANCE OF ASYMMETRIC ACETYLCHOLINESTERASE (AChE) IN CULTURED CHICK MUSCLE CELLS IN THE ABSENCE OF INNERVATION. H. Popiela, C. Flowers*, and B.W. Festoff, Dept. Neurology, University of Kansas Medical Center, Kansas City, Kansas 66103 and Veterans Administration Medical Center, Kansas City, Missouri 64128.

The appearance of asymmetric forms of AChE has generally not been detected in cultured chick skeletal muscle cells in the absence of cocultured neurons. To further explore neurotrophic effects of peripheral nerve extracts on muscle in vitro, we re-examined the appearance of various molecular forms of AChE in cultured chick muscle cells in the presence of adult peripheral nerve extract (NE). The various molecular forms of AChE were distinguished by sensitive sucrose gradient sedimentation and radioenzymatic techniques. Muscle cells were dissociated from 11d old chick embryo leg muscle and filtered through polyester cloth with 10 um pores to obtain single cells. These cells were then cultured in Ham's F-12 medium supplemented with 5% horse serum in the presence of 300 ug/ml NE. Cells proliferated during the first 48h of culture, then fused and formed spontaneously contracting myotubes by 6-8d in culture. Total AChE, 5.4S, and 11.5S molecular forms reached activity plateaus by 8d in culture which persisted until cultures were terminated at 21d. Between 1-7d in culture, 19.5S AChE was not detected. However, this asymmetric form abruptly appeared between 7-8d reaching a maximum of 8% of the total AChE and then gradually declined to a level of 2% at 20d. Since 19.5S AChE represented 25% of the total in 11d embryonic muscle tissue, we examined the possible requirement of neuronal presence in culture to attain higher levels of 19.5S AChE. Spinal cord neurons were plated onto 6d muscle cultures and AChE activities were measured between 8-20d. The results showed that 19.5S AChE activity in the presence of spinal cord neurons and NE was no greater than that found in the presence of NE alone. To prevent the development of contractile myotubes, 0.6 uM tetrodotoxin (TTX) or 15 uM d-tubocurarine (dtC) were added to 5d old parallel muscle cultures at a time when myotubes were differentiated but contractile activity had not begun. TTX inhibited further development of myotubes and also the appearance of 19.5S AChE. However, dtC had no visible effect on morphological development, virtually eliminated contraction, but did not interfere with the appearance of any forms of AChE including the 19.5S form. These studies show that adult peripheral nerve extract promotes the developmental appearance of the 19.5S form of AChE in non-innervated muscle cells in vitro.

54.1 PERINATAL INCREASE OF MOUSE BRAIN ESTROGEN RECEPTORS. W. Friedman, B.S. McEwen, J.L. Gerlach*and D. Toran-Allerand. The Rockefeller University and Columbia University College of Physicians and Surgeons, New York, NY.

Binding of the estrogen, ^{3}H moxestrol, to fetal and neonatal mouse brain cytosol receptors was examined to determine the ontogeny of estrogen receptors in specific brain regions. The areas studied were the hypothalamus and cerebral cortex and the ages ranged from embryonic day 15 to postnatal day 18. Cytosol receptor assays here were performed under exchange condition at 25°C for 4h in order to measure receptors which had become occupied by estradiol during tissue homogenization. Tritiated moxestrol (RU2858 from New England Nuclear) was used at 1nM to selectively label putative receptors and not alpha fetoprotein. Scatchard analysis revealed high affinity (Kd=0.4nM) sites and was in good agreement with single point assays at 1nM, which measured 70% of binding capacity.

Binding was initially examined in the whole forebrain. Total binding of ^{3}H-moxestrol in the forebrain begins to increase between embryonic days 15 and 18 and reaches adult levels at postnatal day 9. The increase in binding relative to protein content peaks at postnatal day 9 and then decreases, whereas the amount of binding relative to DNA content reaches a maximum between postnatal days 12 and 15.

The developmental time course of the estrogen receptors was studied in the hypothalamus and three cortical regions. In the hypothalamus binding of ^{3}H-moxestrol is increasing from postnatal day 5 to day 18. The cingulate cortex shows the highest amount of binding, increasing until postnatal day 9 and then declining. In the other two cortical areas studied, the lateral and posterior cortices, binding, when expressed per mg DNA, is somewhat higher between postnatal days 7 and 15 than in adults. When the binding is expressed per mg of protein there is a dramatic decline after day 7, which is probably a result of a large increase in protein content relative to amount of receptor.

The perinatal period, from fetal day 15 to postnatal day 5, shows a great increase in ^{3}H moxestrol binding in both hypothalamus and cortex. The overall increase in binding is 10-20 fold between E15 and P1, with a more gradual increase between P1 and P5. Thus, estrogen receptor levels, while detectable in the mouse at E15, are subject to a sharp and rapid increase in the perinatal period. This increase may be related to the onset of the phase of sexual differentiation known as "defeminization" which appears to involve estrogen receptors responding to estradiol produced from testosterone and which appears to be confined to the very early postnatal period. (Supported by grants from the USPHS, NSF, Rockefeller Foundation, March of Dimes Birth Defects Foundation and Mellon Foundation.)

54.3 PUBERTY ASSOCIATED NEURAL SYNAPTIC CHANGES IN FEMALE RATS ADMINISTERED ESTROGEN. Richard W. Clough and Jorge F. Rodriguez-Sierra. Dept. Anat., Univ. Nebraska Med. Ctr., Omaha, NE 68105

Estradiol benzoate (EB) administration can induce phasic pituitary gland LH secretion, ovulation and vaginal opening in prepuberal female rats presumably through a neural mechanism. This study investigated whether this effect is associated with changes in synaptic profiles in the mediobasal hypothalamus (MBH) and the medial preoptic area (MPOA). Twenty-five-day-old female rats were administered EB (10 µg/rat, sc, in oil) or EB followed by progesterone on day 27 (P; 1 mg/rat, sc, in oil) or they received no treatment. Blood was collected at 1600 h on day 27 and assayed for LH by radioimmunoassay. Rat brains were perfused and the MBH and MPOA were removed and processed using phosphotungstic acid for selective staining of neural synapses.

Group	N	LH(ng/ml)	MBH Synaptic Vol %	MBH Numerical density X 10^6	MBH Synaptic area density/100 μ^2
Control	5	34± 7	.46±.05	.80±.08	4.39±.26
EB	5	1079±364*	1.11±.03*	1.86±.03*	10.57±.85*
EB + P	5	1771±321*	1.06±.06*	1.78±.11*	9.64±.34*

(* = $p<0.05$ compared to control; mean ± SEM)

Serum LH was markedly elevated in the EB and EB + P treated groups at 1600 h 2 days after EB treatment. This acute rise of serum LH was accompanied by an acute increase of synaptic volume percent and numerical density in the MBH. We observed no such change in the MPOA of EB treated animals compared to controls. We are in the process of investigating other neural areas including the medial septal area and the cortico medial amygdala. Data will be presented for these areas as well as data obtained from treating prepuberal male rats with EB. We conclude from this study that estrogens act through a neural mechanism to accelerate maturation of neuroendocrine processes which govern phasic pituitary gland LH release and that this maturation process entails synaptogenesis in the MBH. (Supported by grants from the Univ. of Nebraska Medical Center, by NIMH grant 36419 and by NIH HD-13219.)

54.2 PREPUBERAL EXPOSURE TO ESTROGEN INCREASES THE NUMBER OF NICOTINIC RECEPTORS IN THE MEDIOBASAL HYPOTHALAMUS (MBH) OF THE FEMALE RAT. J. F. Rodriguez-Sierra, B.J. Morley and R.W. Clough. Dept. Anatomy, Univ. Nebr. Med. Ctr., Omaha, NE 68105 and The Boys Town Institute for Communication Disorders in Children, Omaha, NE 68131.

Our previous work has centered on an experimental model to induce a precocious surge of serum luteinizing hormone (LH) by administration of 10 µg of estradiol benzoate (EB) to a 25-day-old female rat. Two days after EB at 1600h, but not at 1100h, serum LH concentration is elevated 1000 times basal levels. We have previously shown that prepuberal female rats treated with EB incur a dramatic increase in the number of synapses in the MBH when compared to oil-vehicle controls. The present study was conducted to test the possibility that the increased synaptogenesis after EB treatment involves, at least in part, an increase in nicotinic acetylcholine receptors (NAR) in the MBH. Twenty-five-day-old Sprague-Dawley female rats were administered 10 µg of EB or oil vehicle, sc. On day 27 of age, animals were decapitated at 1600h. Trunk blood was collected, centrifuged at 1500 x g for 10 min at 4°C and serum frozen (-20°C) until radioimmunoassay for LH was performed. The brains were removed from the skull, blocked coronally at the level of the optic chiasma-anterior commissure decussation. The brain was sliced at 1 mm sections thick. Sections were placed in ice, transilluminated and the preoptic area (POA), septal area (SEP), MBH and somatosensory cortex (CX) were removed and placed in ice-cold buffer. Tissue was homogenized, centrifuged at 100,000 x g for 30 min, the supernatant was discarded, pellet was resuspended in Triton X buffer and centrifuged again at 100,000 x g for 30 min. Radioreceptor assay for NAR was performed using α-bungarotoxin (BuTX) as the radioligand.

	(N)	BuTX Binding MBH[a] (fmoles/mg protein
EB	14	89.4±10.5*
Oil	12	62.8± 7.6

[a]Mean ± SEM; *$p<0.05$.

The EB-treated rats exhibited a significant increase in the number of BuTX binding sites in the MBH. The POA and SEP showed a small, but non-significant increase of BuTX binding, while the CX was unaffected by the treatment. The results point to acute morphological and neurochemical changes in the MBH due to EB treatment in prepuberal female rats and suggest that the onset of puberty is partially due to an increase in the number of cholinergic synapses. Supported by grants from NSF (AO-06648) to B.J.M. and NIH (HD-13219) to J.F.R-S.

54.4 PRENATAL EXPOSURE TO ESTROGEN MASCULINIZES AND DEFEMINIZES BEHAVIOR IN THE GUINEA PIG. M. Hines, P. Alsum*, R.A. Gorski & R.W. Goy. Dept. of Anatomy & Lab. of Neuroendocrinology, Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA, and Wis. Reg. Primate Res. Ctr., U. of Wisconsin, Madison, WI.

Genetic female rats and hamsters treated with estrogen during perinatal critical periods of development show enhanced masculine copulatory behavior (masculinization) and impaired feminine copulatory behavior (defeminization). In these species critical developmental periods are largely postnatal, and it is not known if estrogen has similar effects in species, such as the guinea pig, in which critical periods occur prenatally. Therefore, we treated guinea pigs with estradiol benzoate (EB) or diethylstilbestrol (DES), a synthetic estrogen that is more potent than estradiol, from day 29 to day 68 of pregnancy. Hormones were injected subcutaneously in 0.1 ml oil daily for the first six days and on alternate days thereafter. Doses and the number of female offspring in each treatment group were: 1 µg EB (n=8), 2 µg EB (n=7), 3.3 µg EB (n=8), 1 µg DES (n=4), 3 µg DES (n=6), oil (n=4), and untreated (n=13). Animals were ovariectomized as adults and tested twice for feminine behavior following priming with EB and progesterone, and twice for masculine behavior without hormone replacement. For feminine behavior, the duration of the lordosis response to stroking was assessed hourly for 12 hours following hormone priming. For masculine behavior, a stimulus female was introduced into the home cage for 15 minutes and the number of correctly oriented mounts was recorded. Animals treated with 3 µg DES were clearly masculinized and defeminized. Compared to controls, more of these animals mounted (83% vs. 13%, $p<0.001$), and they mounted more frequently (4.8±2.5 vs. 0.1±0.2, $p<0.01$). In addition, fewer of them showed a lordosis response (50% vs. 100%, $p<0.01$) and the quality of their response was impaired. The peak duration was shorter than controls (1.6±0.7 vs. 11.4±0.7 secs, $p<0.001$) as was the average duration for all responses to stroking (0.8±0.3 vs. 5.7±0.7, $p<0.001$). Similar but less dramatic effects were seen in animals treated with 1 µg DES. Although 75% of these animals showed lordosis, the peak and average durations (6.8±1.9, 3.3±0.8 secs, respectively) were less than in controls ($p<0.01$, $p<0.05$, respectively). In addition, more of these animals than controls mounted (75%, $p<0.05$) and they mounted more often (4.4±3.3, $p<0.01$). There was also some evidence of masculinization in terms of mounting frequency in animals treated with 3.3 µg EB (0.6±0.4, $p<0.05$). These results indicate that estrogen-induced masculinization and defeminization are not limited to animals in which critical periods for sexual differentiation occur postnatally. Supported by NIH grants HD00182, RR00167, MH21312 and NS6594.

54.5 DIFFERENTIAL EFFECTS OF THE PERINATAL STEROID ENVIRONMENT ON TWO PARAMETERS OF SEXUAL DIFFERENTIATION. R. J. Handa*, J. E. Shryne*, J. N. Schoonmaker*, P. Corbier* and R. A. Gorski. Dept. of Anat. and Brain Research Inst., UCLA School of Medicine, Los Angeles, CA 90024 and Lab. d'Endocrinologie, Universite Paris-XI, Orsay.

Previous studies suggest that the presence of a post-partum testosterone surge in male rats may be of importance in the suppression of the development of feminine sexual behavior. In this study we have examined the influence of the perinatal steroid milieu on two parameters of sexual differentiation: the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA) and the positive feedback response to estrogen (E) and progesterone (P) treatment. Sherman rats were divided into groups according to sex and time of gonadectomy (Gx). Females were either Gx'd at 0 h (i.e. in utero), or sham operated and Gx'd at 29 days of age. Males were Gx'd at 0 h, or 6-7, 12-13 or 24 h post partum or sham operated at 0 h followed by Gx at 29 days of age. In addition, another group of males Gx'd at 0 h were injected with 5 µg testosterone propionate (TP) sc immediately after surgery. The induction of a positive feedback response of LH to E and P was performed between 100-120 days of age. Blood was taken by jugular puncture at 29 and 53 h following E treatment and 5 h after P treatment and assayed for LH by RIA. Two weeks later rats were sacrificed and perfused for analysis of SDN-POA volume. In males, a significant ($p<0.001$) increase in the amplitude of the LH surge was exhibited by animals Gx'd at 0 h as compared to those of males Gx'd at later time periods. Treatment with 5 µg TP did not change the amplitude of the LH surge but decreased its incidence. Also, the incidence of the LH surge decreased with time of postnatal Gx from 50% at 0 h to 14% at 24 h. No sham Gx'd males showed an LH surge. In females there was a significant ($p<0.001$) change in the amplitude of the LH surge between those Gx'd at 0 h and 29 days (shams). Furthermore, the amplitude of the surge shown by 0 h females was comparable to those of 0 h males. SDN-POA volume was found to be unchanged in all perinatally Gx'd males but were significantly ($p<0.001$) different when compared to that of sham operated males and females. Treatment with 5 µg TP increased by 50% ($p<0.001$) the size of the SDN-POA of males Gx'd at 0 h, but did not completely restore it to that seen in sham Gx'd males. In addition, a decrease ($p<0.05$) in SDN-POA volume was noted in 0 h Gx'd females when compared to that of sham Gx'd females. Thus, 1) the postpartum testosterone surge is important in the differentiation of LH surge mechanisms but it is without an obvious effect on the development of the SDN-POA. 2) 5 µg TP given immediately after parturition influences the development of the SDN-POA as well as LH mechanisms. 3) In the female rat the ovary may be of importance in normal female sexual development. Supported by AG 01754, GM 07191 and HD 01182.

54.6 ANALYSIS OF THE PATTERNS OF GROWTH HORMONE SECRETION IN TESTICULAR-FEMINIZED (tfm) RATS. W.J. Millard*, J.A. Politch, J.B. Martin, T.O. Fox. Dept. of Neurology, Massachusetts General Hospital, Dept. of Neuroscience, Children's Hospital Medical Center, Harvard Medical School, Boston, MA 02115.

We have previously demonstrated that the sexually dimorphic patterns of growth hormone (GH) secretion in adult rats are not entirely dependent upon the gonadal steroid environment during the early postnatal period. Neither castration of neonatal males nor androgenization of neonatal females resulted in a complete alteration of the respective homotypic GH patterns. Since the development of some behaviors in the rat is affected by steroids during prenatal development, we have begun to investigate whether prenatal "organizing" effects of gonadal steroids influence the GH secretory patterns in adult animals. We examined an androgen-resistant mutant rat, testicular feminization (tfm), maintained as a Stanley-Gumbreck line derived from a King-Holtzman stock. These animals are deficient in androgen receptors and many androgen-dependent characteristics. We compared the GH secretory patterns in these mutants with their normal male and female siblings. If prenatal exposure to steroids is responsible for the adult masculine pattern of GH secretion, then tfm animals should exhibit the female GH secretory profile. All animals, 6-8 months of age, were prepared with intraatrial silastic catheters, and following recovery from surgery, placed in specially designed sampling chambers 48-72 hrs prior to experimentation. Animals were bled via the indwelling catheter every 15 min for 8 hr (900-1600 hr) and the GH levels in the plasma determined by RIA. Normal males displayed the characteristic "episodic" pattern of GH secretion with bursts of GH (values usually > 300 ng/ml) occurring every 3-4 hr and separated by GH trough (values usually < 5 ng/ml) periods of 30-90 min in duration. Females showed an irregular pattern of GH secretion with "pulses" of GH occurring every 1-2 hr; the individual GH peak heights were lower (values < 150 ng/ml) and the GH trough values (10-20 ng/ml) higher than those of normal male animals. The tfm animals displayed GH secretory profiles which resembled those of their normal female littermates, i.e. increased frequency of GH bursts, diminished GH pulse amplitudes and elevated GH trough levels relative to normal males. These data are consistent with the hypothesis that the gonadal steroid environment during the prenatal period of development plays a major role in determining the sexually dimorphic patterns of GH secretion in the rat. Since tfm rats are deficient in androgen receptors, androgen might serve in this capacity. We have not ruled out, however, that non-steroidal factors are involved in the expression of female-like GH patterns in tfm rats.

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54.7 EFFECTS OF PRENATAL EXPOSURE TO FLUTAMIDE ON FEMALE SEXUAL BEHAVIOR AND MUSCARINIC BINDING IN MALE RATS. J. A. Witcher*, G. P. Dohanich and L. G. Clemens. Department of Zoology, Michigan State University, East Lansing, MI 48824.

Sexual differentiation of the rat brain is dependent upon the hormonal environment that prevails during prenatal and early postnatal life. Exposure to perinatal androgen may decrease the ability to respond to estrogen in adulthood. For example, estrogen regimens that activate a high level of female sexual behavior in ovariectomized female rats are usually less effective in activating this behavior in castrated males. Estrogen also has the capability of altering the number of cholinergic muscarinic binding sites in the preoptic and hypothalamic areas of female rats but not of male rats. This sex difference in the potential of estrogen to affect cholinergic activity may have important biological implications since recent evidence indicates that cholinergic transmission in the female rat brain contributes to the regulation of the lordosis response, an estrogen-dependent sexual behavior. An experiment was conducted to determine if male rats exposed prenatally to the antiandrogen, flutamide, would display behavioral and physiological responses to estrogen that are typically feminine in character; specifically, enhanced levels of female sexual behavior and significant changes in muscarinic binding following estrogen treatment in adulthood.

Long-Evans male rats were exposed to flutamide (5 mg/day/mother) or propylene glycol vehicle on days 10-21 of gestation. In adulthood, randomly-selected males (90-100 days of age) were gonadectomized and three weeks later were injected with either estradiol benzoate (10 µg/kg, i.m.) or sesame oil (0.1 ml, i.m.) for three days. Muscarinic binding was analyzed in the medial preoptic area, medial basal hypothalamus, septum, and parietal cortex using a tritiated antagonist, 3-quinuclidinyl benzilate. Male littermates were treated with estradiol benzoate (10 µg/kg for three days, i.m.) and tested for lordosis behavior elicited by stimulus male rats. Although male littermates given prenatal flutamide displayed enhanced lordosis behavior in response to estrogen treatment when compared to propylene glycol controls, no significant changes in muscarinic binding were detected following estrogen treatment in any brain area analyzed. Consequently, prenatal flutamide feminized the behavioral response of males to estrogen without altering the inability of males to exhibit estrogen-induced changes in muscarinic binding. It is suggested that behavioral feminization arising from prenatal exposure to flutamide is not directly correlated to measurable changes in muscarinic binding within the male brain.

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54.8 SEX DIFFERENCE IN DENDRITES DURING DEVELOPMENT OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA. Ronald P. Hammer, Jr. and Carol D. Jacobson. Lab. of Neurophysiology, NIMH, Bethesda, MD 20205 and Dept. Vet. Anatomy, Iowa State University, Ames, IA 50011.

In the Sprague-Dawley rat, the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA) is larger in volume in the male sex. Results from the present study indicate that by day 10 of postnatal life (PN) the dendritic systems of neurons in the male SDN-POA are more extensive than those of females both in length and pattern of branching. At birth, the dendritic arborizations are similar in the male and female. However, rapid dendritic growth in the male ensues between day 4 PN and day 6 PN. All animals utilized in this study were obtained from lactating females which were bred in the animal colony. A sperm-positive vaginal smear on estrus was defined as day 1 postfertilization (PF). All females delivered on day 23 PF, defined as day 1 PN. Male and female pups were sacrificed by decapitation on either day 2, 4, 6 or 10 PN. Dendritic status was assessed using the Stensass modification of Golgi staining technique. Analysis of Golgi impregnated SDN-POA neurons involved measuring dendritic expanse and number of branches as well as dendritic spines. Somata contained in the region characterized as the SDN-POA are rounded in the neonatal animal. However, they become more elongated during development. These neurons generally extrude three or four primary dendritic branches, the number of which remains constant during early postnatal development. Further branching of the dendritic arbor occurs with the formation of secondary and tertiary dendrites. Rarely does branching beyond this occur prior to day 10 PN. Growth regions on the dendrites are evidenced by varicose regions which are more prominent on terminal branches. SDN-POA neurons are often spiny. The appearance of these spines, which represent further development of the neuropil, is greater in the male after day 4 PN and after day 6 PN in the female. The increase in the male dendritic array after day 4 PN coincides with the previously observed sexual differentiation of the volume of the SDN-POA. These data suggest that the sexual differentiation of the SDN-POA may in part be due to the changing neuronal morphology which is taking place during the time period when gonadal steroids are capable of influencing the resulting adult volume of the SDN-POA. (Supported in part by NIH grants RR07034 and HD-16148).

54.9 NEUROGENESIS OF MOTONEURONS IN THE SEXUALLY DIMORPHIC SPINAL NUCLEUS OF THE BULBOCAVERNOSUS IN RATS. C.L.Jordan, S.M.Breedlove & A.P.Arnold. Psychology Department, UCLA, Los Angeles, CA 90024.

The spinal nucleus of the bulbocavernosus (SNB) consists of motoneurons innervating striated perineal muscles in male rats. Motoneurons in the SNB region are only one-third as numerous in females as in males (Breedlove & Arnold, Science 210 564, 1980), and the number of motoneurons can be increased or decreased by perinatal manipulations with androgen or anti-androgen. It is not yet known whether such manipulation of the number of SNB cells is due to alteration of the proliferation, death or specification of motoneurons. We now report results from thymidine autoradiography indicating that SNB motoneurons are post-mitotic by the 15th day of gestation. Since hormonal manipulations more than a week later can increase or decrease the number of SNB cells, such manipulations are probably mediated by alterations in the death or specification of cells, but not their proliferation.

Fetal Sprague-Dawley rats were exposed to tritiated thymidine via a single ip injection of pregnant dams (5 uCi/g body weight, 45 Ci/mmol) on one of 14 days of gestation (days 9-22) or sc injection of rat pups on either gestational day 23 (day 1 of life) or day 3 of life. All animals were sacrificed in adulthood (day 57) and the lumbar-sacral spinal cords were prepared for autoradiography. After 9 weeks of exposure, SNB cells were considered unlabeled if they had 6 or fewer silver grains over the nucleus, lightly labeled if they had 7 to 11 grains, and heavily labeled if they had 12 or more silver grains over the nucleus. More than 90% of SNB cells incorporate thymidine on gestational day 12, but SNB motoneuron nuclei were never heavily labeled in rats injected with thymidine on the 15th day of development or later, thus indicating the completion of neurogenesis. The table below describes the labeling of SNB cells in males, the pattern of which seems identical in females, despite the scarcity of SNB cells in adult females.

DAY INJECTED	- d9	d10	d11	d12	d13	d14	d15 & later
LIGHTLY LABELED-	28.6%	32.2%	22.8%	17.1%	0%	0%	0.4%
HEAVILY LABELED-	11.4%	5.5%	10.1%	75.0%	4.0%	2.9%	0%
number of cells	(70)	(90)	(79)	(76)	(100)	(70)	(687)

Because appropriate hormone treatment more than a week after the end of mitosis can either increase or decrease the adult number of SNB cells, such alterations probably are due to effects on motoneuronal death rather than proliferation. By extension, the normal development of a sexually dimorphic number of SNB cells is also probably due to differential cell death.

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54.10 ESTRADIOL INCREASES NUMBERS OF NEURONS AND SYNAPSES IN NEONATAL RAT SUPERIOR CERVICAL GANGLION. L. L. Wright and A. J. Smolen, Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Neonatal treatment with gonadal steroids has been reported to alter morphological as well as functional development in various regions of the brain and spinal cord. These regions have been found to be sexually dimorphic, and the gender differences in neuron and synapse numbers dependent upon the level of gonadal steroids during the perinatal period. It has been shown previously that neonatal treatment with testosterone propionate (TP) results in an increase in the number of neurons in the superior cervical ganglia (SCG) of female (Dibner & Black, J. Neurochem. 30:1479-1483, '78) and male (Wright & Smolen, Soc. Neurosci. Abst. 7:5, '81) rats. Since there are normal gender differences in circulating levels of testosterone perinatally which could influence neuron numbers, we compared ganglia of adult male and female rats. We found that the males have 20% more SCG neurons than the females. In some sexually dimorphic CNS regions, estrogen has been found to be the active metabolite of testosterone responsible for gender differences. We therefore investigated the effects of estrogen on the developing SCG neurons and synapses. 17-b-estradiol (E_2) (.05 µg/g) was suspended in oil and administered subcutaneously on alternate days from the day of birth until the time of sacrifice on day 15. Thus, treatment continued throughout the period of normal neuron death (Wright et al. Anat. Rec. 199:217A, '81), and during the time when most synaptogenesis occurs (Smolen & Raisman, Brain Res. 181:315-323, '80). Treatment with E_2 results in a 95% increase in neurons. Studies in progress will determine whether this increase in neuron numbers is due to increased proliferation or decreased neuron death. Synaptogenesis was also altered by estradiol treatment, resulting in a 130% increase in SCG synapses.

Gonadal steroids may modulate neuron and synapse numbers by direct action on receptors within the SCG. Alternatively, the steroids could act at receptors on targets or afferents of the SCG, altering neuron and synapse development by changing the interactions with these cells. Circulating testosterone, possibly via local metabolism to estrogen, may mediate the sex difference in SCG neuron numbers, as it does in sexually dimorphic regions of the CNS. Gonadal steroids may also play a role in the normal development of SCG neuron and synapse populations in both genders, since low levels of estrogen and testosterone are present during the perinatal period in males and females.

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54.11 SEXUAL DIFFERENCES IN 2-DEOXY-D-GLUCOSE UPTAKE IN THE BRAINS OF DEVELOPING RATS. C. L. Kornblith, W. W. Youngblood*, and J. S. Kizer*. Biol. Sci. Res. Ctr., Univ. North Carolina Sch. of Med., Chapel Hill, NC 27514.

Structural sex differences have been found in the central nervous system, especially in avian vocal control areas (Nottebohm and Arnold, Science, 194: 211, 1976) and in rodent preoptic area (Gorski et al., Brain Res., 148: 333, 1978; Greenough et al., Brain Res., 126: 63, 1977) and the spinal nucleus of the bulbocavernosus in the lumbar spinal cord (Breedlove and Arnold, Science, 210: 564, 1980). The participation of these structures in courtship or sexual behavior has been demonstrated.

We felt that additional brain regions that are less directly involved in hormonal actions and sexual behavior might also display sexual differences. Such differences could be represented morphologically and/or in functional activity. Furthermore, these differences, particularly in functional activity, might be present only at certain times during development.

In order to locate areas with sexually dimorphic functional activity, we measured the uptake of ^{14}C-2-deoxy-d-glucose (2DG) in the brains of male and female rats of different ages. Eight peripubertal (33 to 37 days of age) and eight adult rats were injected i.p. with 125 uCi/kg of 2DG and returned to a familiar environment. Following a 45 min period during which behavioral observations were recorded at 30 sec intervals, the animals were sacrificed and the brains were prepared according to the autoradiographic method described by Sokoloff et al. (J. Neurochem., 28: 897, 1977). Eight coronal sections from each brain were selected for analysis. A computer-aided, television-based system was used for densitometric measurements. Grey to white matter ratio values for specific areas were averaged across animals of the same sex and age. Between group differences were then examined.

We found very interesting differences in the patterns of functional activity at all levels of the neuraxis as a function of both age and sex. Behavioral differences during the test could not account for differences in functional activity. For both sexes, there was generally greater 2DG uptake in the adult than in the peripubertal animals. Adult proestrus females showed the largest difference in labelling compared to young females. In the young animals there were few significant differences in functional activity between the sexes. For the adults, complex sex differences in the pattern of 2DG uptake were found at several levels of the brain, and varied as a function of the phase of the estrus cycle in the females. Our results suggest that the differences between male and female brains are greater than indicated by previous studies. Supported by HD-14005 and MH-00114.

54.12 ESTROGEN AND PROGESTIN RECEPTORS IN FETAL RAT HYPOTHALAMIC TRANSPLANTS GROWN IN GONADAL STEROID-FREE ADULT HOSTS. C. M. Paden, J. L. Gerlach* and B. S. McEwen. The Rockefeller University, 1230 York Avenue. New York, NY 10021.

Determining the factors which govern the appearance of hypothalamic estrogen receptors during the perinatal period is important in understanding the mechanisms of sexual differentiation in the rat. We have investigated the influence of gonadal hormones on the appearance of estrogen and progestin receptors in fetal rat hypothalamus transplanted into adult host brains. This technique allows for manipulation of the hormonal environment of the developing brain to an extent which is impossible in the fetus in situ.

Fetuses were removed from Charles River CD rats under barbiturate anesthesia on days 15-18 of gestation. Hypothalamic transplants were made onto the choroidal pia overlying the superior colliculus in young adult female CD hosts as previously described (Stenevi, U., et al., Cell Tiss. Res., 205:217, 1980). Hosts were (a) intact or (b) ovariectomized (OVX) and adrenalectomized (ADX) 72 hours previously or (c) OVX-ADX and implanted sc with a 1 cm silastic capsule of estradiol (E_2). E_2 implants were removed 4 weeks later and all hosts were decapitated 8 weeks after transplantation. Estrogen and progestin receptors were assayed in transplant cytosol (MacLusky, N. J. and McEwen, B. S., Brain Res. 189:262, 1980). Saturable binding of radiolabeled E_2 to cytosol protein was found in all hypothalamic transplants. This binding exhibited the steroid specificity of authentic estrogen receptors. Scatchard analysis was linear, giving a K_d of 0.26 nM and B_{max} of 7.12 femtomoles/mg protein. No significant differences in the level of estrogen receptors were observed in transplants from groups a, b or c. In addition, no differences in levels were observed in transplants taken from male or female fetuses on gestational days 17 or 18. Day 15 and 16 fetuses were not sexed. Concentration of ^{3}H-E_2 by transplant neurons in vivo was also demonstrated using autoradiography. No differences in the pattern of concentration between transplants from intact and OVX-ADX hosts were found, and indeed transplants were indistinguishable from host hypothalamus. Saturable binding of ^{3}H-promegestrone to progestin receptors was also detectable in transplants from both intact and OVX-ADX hosts. Progestin receptor levels were significantly increased in both groups when hosts were given 2 sc injections of 10 µg estradiol benzoate 72 and 48 hours before death.

We conclude that exposure to gonadal steroids during either the last week of gestation or the postnatal peroid is not required for the elaboration of hypothalamic estrogen and progestin receptors in the rat. Furthermore, the estrogen receptors which appear under gonadal steroid-free conditions are functional as determined by the ability of estradiol to induce progestin receptors in hypothalamic transplants.

55.1 INNERVATION OF PERIPHERAL NERVE SEGMENTS GRAFTED INTO RAT OLFACTORY BULB. B. Friedman and A. Aguayo, Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4

Using retrograde axon tracing methods it has been recently demonstrated that in the mature mammalian central nervous system, certain neurons grow axons into peripheral nerve segments grafted into the brain or spinal cord (Aguayo et al. In, Advances in Cellular Neurobiology 3:215-234, 1982). It is not known if processes of all types of central neurons will grow into such grafts. The olfactory bulb is a convenient site to study whether large projection neurons (mitral cells) and related small interneurons (periglomerular, granule and short-axon cells) have the capacity to innervate a peripheral nerve graft. The somata of the bulbar projection neurons and interneurons occupy separate lamina and possess distinct morphologies. Among the interneurons, the granule cells are further distinguished by (i) their great number and (ii) their apparent lack of axonal processes.

This study examines the capacity of these various bulbar cell types to grow axons into autologous peroneal nerve segments which have been transplanted into the surgically exposed dorsal surface of the olfactory bulb(s) in adult male Sprague-Dawley rats. The distal portion of the graft is placed extracranially and the distal tip either sutured to the ipsilateral temporalis muscle or inserted into the contralateral olfactory bulb. From 6-8 weeks after grafting , a pledget of gelfoam soaked in 20% HRP was placed onto the extracranial stump of the transected nerve graft. The cut was surrounded by vaseline and parafilm to avoid tracer diffusion.

In cases where there was good fusion of the graft to the olfactory bulb, the tip of the graft was typically located in the granule cell layer. HRP application to the graft produced retrogradely labelled profiles which were identified as mitral cells on the basis of their laminar position, shape and size. To date, none of the bulbar interneurons have been labelled. These findings suggest that bulbar projection neurons can grow into peripheral nerve grafts while the bulbar interneurons apparently lack this capacity. However, it is also possible that the HRP tracing method may be insufficiently sensitive to detect small bulbar interneurons which may have innervated the graft.

55.2 TURNOVER OF OLFACTORY RECEPTOR PROTEINS. R. Schafer and J.C. Dickens*. Dept. Biol. Sciences, North Texas State Univ., Denton.

It has been generally assumed that odorant receptor sites in olfactory receptor membranes are not altered irreversibly or "used up" when they interact with odorants during the transduction process. However, this assumption has never been tested experimentally. Furthermore, the rate of turnover (degredation/replacement) of olfactory receptor molecules has not been measured. Experiments are underway using protein synthesis inhibitors in an attempt to discover the life span of olfactory receptor molecules. These experiments involve long-term recording from the frog olfactory mucosa after application of certain inhibitors. One side of the nose is exposed to an inhibitor in perfusion solution, while the other side serves as a control which is exposed only to the perfusion solution.

Topical application of puromycin, a powerful inhibitor of protein synthesis, inhibits the incorporation of radioactively-labeled amino acids into protein (TCA-insoluble material) in the olfactory mucosa. Experiments so far have achieved a level of about 80% protein synthesis inhibition without affecting electrophysiological responses to odorants over a period of many hours. The tentative conclusion (which agrees with the original assumption) is that olfactory receptor molecules are not "used up" during the transduction process. The experiments so far indicate a receptor half life in excess of eight hours, even in the face of continuous stimulation at one minute intervals.

Vaporous alkylating agents (chemically active odorants) are also being used in this study in a different approach to the problem. In this case, chemically active odorants such as ethylbromoacetate are used to chemically alter and physiologically inactivate the olfactory receptor proteins at the cell surfaces. Recovery of electrophysiological responsiveness should occur as the receptors are regenerated by protein synthesis. Preliminary results using this technique indicate that recovery (if it is possible after such treatment) is a slow process which requires a time course on the order of many hours. (Supported by NSF grant BNS-81-08842 and NTSU Faculty Research Grant 34682 to R.S.)

55.3 DYNAMICS OF OLFACTORY MARKER PROTEIN (OMP) TURNOVER AND TRANSPORT IN MICE. R. M. Kream* and F. L. Margolis, (SPON: E. Cantor) Roche Institute of Molecular Biology, Nutley, N. J. 07110

OMP is a major cytosol protein of 19,000 daltons that is a cell-specific marker of mature olfactory chemosensory neurons. OMP may be labelled in vivo to high specific activities (2000 to 3000 dpm/µg OMP) by intranasal irrigation of mice with [^{35}S]-methionine, [^{3}H]-lysine or [^{3}H]-leucine (Kream and Margolis, Amer. Soc. for Neurochem., Abst. 315, 1982). We now report significant differences in OMP turnover and transport between young and old mice. Two groups of CD-1 female mice, one adult ex-breeder and the other 5-6 weeks of age received 300uCi of [^{35}S]-methionine by bilateral intranasal irrigation at a final concentration of 100µM and specific activity of 50Ci/mmol. Olfactory bulbs and epithelia (epi) were dissected separately from each animal, homogenized and high-speed supernatants were prepared from these homogenates. Acid- precipitable and non-volatile acid- soluble radioactivity was measured. OMP was immunoprecipitated by goat anti-OMP, and immunoprecipitates were separated on exponential gradient SDS gels. Proteins were visualized by a silver-stain and quantitated on a laser scanner. Radioactivity in the OMP band was released by digestion with 15% H_2O_2, and measured by liquid scintillation spectrometry. In adult animals, OMP specific activities (OSA) peak between 1 and 2 days in the epi and at 5 days in the bulb, whereas in young animals, OSAs peak at 4 hr in the epi and at 2 days in the bulb. In adults, OSAs are virtually identical to global cytosol protein specific activity values (CSA) in the epi. By contrast, in young animals OSAs are at least 2-fold higher than CSA values in the epi at early time points. Whereas OSAs are normally 4-5fold higher than CSAs in adult bulbs, in young bulbs these values are 5-8fold higher. In the adult group, OSAs in the bulb begin to exceed OSAs in the epi after 4 days. In young animals, OSAs in the bulbs begin to exceed epi values at 2 days. We conclude that these major differences in OSA values vs. time between age groups demonstrate an accelerated rate of synthesis of OMP in young animals. In both young and old animals, OSAs undergo first-order decline in the epi between 2 and 28 days with apparent $t_{1/2}$s of 5-7 days. However, the $t_{1/2}$ for decline of OSA in young bulbs is 6 days vs 7-10 days in adult bulbs. Thus, the accelerated rate of synthesis of OMP in the epi of young animals is matched by an accelerated rate of degradation in the bulb. We have also measured first-order decline of [^{35}S]-methionine vs. time in extracts of epi and bulbs. In all cases, the specific activity of [^{35}S]-methionine in OMP exceeds that of the free amino acid several fold. This indicates minimal reutilization of labelled methionine. Thus, both the maximum specific activity of OMP, its rate of turnover and speed of entrance into the bulb are elevated in young vs. mature mice.

55.4 EXOGENOUS β-ALANINE ELEVATES OLFACTORY TISSUE CARNOSINE LEVELS IN VIVO AND IN VITRO. T. Kawano*, M. Grillo* and F. L. Margolis Roche-RIMB, Nutley, N. J. 07110, A. Farbman, Dept. Neurobiol. & Physiol., Northwestern Univ., Evanston, Illinois 60201, (SPON: R. Wurzburger).

Carnosine (β-ala-L-his) is present in the olfactory tissue of many vertebrates where it is localized in the chemoreceptor neurons. This dipeptide is enzymatically synthesized from its component amino acids by carnosine synthetase. Under normal, physiological conditions, the tissue concentration of histidine is adequate to saturate the enzyme. Reduction of tissue histidine levels subsequent to consumption of histidine-deficient diets, results in reduced tissue carnosine levels. (Quinn & Fisher, J. Neurochem. 29, 717 [1977]). In contrast, normal tissue concentrations of β-alanine are well below the Km of the synthetase for this substrate. Therefore, we evaluated the impact of increasing tissue β-alanine levels on the content of carnosine in olfactory tissue at various ages, both in vivo and in vitro. Carnosine synthesis begins prenatally in the olfactory tissue of the rat and can first be demonstrated in vitro by incorporation of ^{14}C-β-ala on embryonic day 14 and chemically on embryonic day 16. Dosing pregnant females with β-alanine for two consecutive days results in elevations of olfactory tissue carnosine content. Thus, at 18 days of gestation, controls and β-ala-dosed fetuses contain 10 and 100 pmoles/mg tissue respectively. At 20 days gestation, the equivalent values are 20 and 200. Treatment with β-alanine for several days prior to death resulted in a several-fold elevation of carnosine levels in olfactory bulbs of 1-week old and adult rats. Thus, this phenomenon is exhibited at all ages in vivo. As an extension of these studies, embryonic olfactory epithelium was maintained in organ culture in medium supplemented with 1mM β-alanine. This treatment resulted in the precocial appearance of the developmental increase of tissue carnosine. Taken together, these observations demonstrate that exogenous β-alanine has a significant influence on tissue carnosine content both in vivo and in vitro. Possible mechanisms underlying this phenomenon, are: 1) normal tissue β-alanine levels are below the synthetase Km, 2) β-alanine induces synthetase activity, 3) β-alanine inhibits carnosinase activity. Any of these would result in net carnosine accumulation. The levels of acetylcholine, catecholamines and indoleamines have been reported to be elevated by increased levels of their precursors (Wurtman et. al., Pharmacol. Revs. 32, 315 [1980]). However, the magnitude of the effect of β-alanine on carnosine levels is much larger than in those cases. In addition, we believe carnosine is the first peptide to be so regulated.

55.5 AXONAL ENDINGS OF THE CHORDA TYMPANI AND THE LINGUAL (TRIGEMINAL) NERVE BOTH SYNAPSE IN THE SOLITARY NUCLEUS OF THE HAMSTER. M. Whitehead, B. Kinsella*, and M. Frank. Dept. of Oral Biology, University of CT School Dental Medicine, Farmington, CT 06032.

The chorda tympani and the lingual nerve both innervate the anterior tongue but they mediate different sensations. The chorda tympani is a peripheral branch of the facial nerve which primarily mediates taste; the lingual nerve is a trigeminal branch which subserves primarily pain, temperature, and touch. The connections of these two nerves with the medulla have been in the hamster by cutting either the chorda tympani or the lingual nerve in the cheek and exposing the central stump to HRP. In some animals the chorda tympani was labelled in the middle ear to maximize the Golgi-like filling of afferent axons and to rule-out spread of the enzyme to trigeminal branches. Conversely, to limit HRP to the trigeminal nerve, in other experiments, the lingual nerve was labelled after excising the chorda tympani from the middle ear.

Fibers of the chorda tympani enter the medulla ventral to the vestibular nerve and travel obliquely, dorsomedially through the spinal tract of the trigeminal nerve to reach a point just rostral to the solitary nucleus and dorsolateral to the superior salivatory nucleus. From this point the axons extend caudally through the lateral solitary nucleus, join the solitary tract, and send thin and varicose terminal branches into the nucleus. These axons are oriented in the mediolateral plane and terminate in both the dorsomedial and dorsolateral parts of the solitary nucleus. Fibers of the chorda tympani synapse as far caudally as the level of the area postrema although they are most numerous rostrally. A few labelled fibers travel caudally in the spinal tract of the trigeminal nerve and terminate sparsely adjacent to and within the rostral and caudal extremes of the spinal nucleus of the trigeminal nerve.

Lingual nerve fibers project to both the lateral subdivision of the solitary nucleus at the midpoint of its rostral-caudal extent, and to the dorsal one-third of the spinal nucleus of the trigeminal nerve throughout its extent. Lingual terminals in the solitary nucleus are densely distributed and overlap those of the chorda tympani. Thus peripheral nerves subserving taste and touch both from the anterior tongue synapse in the lateral portion of the solitary nucleus. Gustatory response properties of some neurons in the lateral solitary nucleus suggest a convergence of primary afferent inputs mediating different taste qualities (Travers and Smith, Sensory Processes, 1979). It remains to be seen whether these neurons may also respond to tactile and thermal aspects of gustatory stimuli.

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55.6 HEPATIC AND GUSTATORY INTERACTIONS IN THE BRAINSTEM OF RAT G.E. Hermann and R.C. Rogers, Northwestern Univ. Sch. Med., Chicago, IL 60611.

Visceral and gustatory afferents have been implicated in the regulation of physiological and behavioral water and nutrient balance. Indeed, several investigators have found that visceral and gustatory inputs appear to coregulate ADH release, drinking and feeding behavior. Past studies by our group have indicated that this coregulation of functions may be based on overlapping, even convergent, central projections of hepatic and gustatory afferents. We have established, in a series of combined electrophysiological and peroxidase histochemical studies, that the first order projections of hepatic and gustatory afferents to the n. tractus solitarius (NTS) are quite separate and show no evidence of physiologic overlap. Additionally, the hepatic vagal afferent projects mainly upon the left NTS. Very small (100-200u in D), iontophoretic injections of HRP into physiologically-identified zones of the NTS reveal that the immediately subjacent parvocellular RF both sends and receives projections from both the hepatic and gustatory division of this nucleus. Furthermore, both NST divisions send projections to the parabrachial nucleus which converge posteriorly and diverge anteriorly.

These results will form a basis for future studies of visceral afferent alteration of perceived gustatory quality, visceral afferent switching of taste preference behavior and viscero-gustatory coregulation of physiologic water balance. Additionally, these results provide direct anatomical evidence for the observations of Rogers, et al of hepato-gustatory coactivation of neurons in the parabrachial nucleus (J. Autonom. Nerv. Sys., 1: 183-202, 1979). (Supported by the Bane Research Fund, NUMS research committee and NINCDS grant NS 17135 to RCR)

55.7 TASTE RESPONSES IN THE AMYGDALA. Joel R. Morse, Department of Psychology, SUNY at Stony Brook, Stony Brook, NY 11794.

The amygdala has been linked to the control of a variety of survival behaviors and functions. In keeping with this role data indicate that the amygdala samples information from internal and external milieu, including visceral afferent and general sensory information. Sensory responses in all modalities have been recorded from amygdala units with, until recently, the exception of taste. Anatomical and electrophysiological findings in the rat (Norgren, 1976) indicated that gustatory neurons might be found in the amygdala and initial observations in the rabbit proved this to be so (Schwartzbaum and Morse, 1978).

To extend these initial observations units were sampled from the amygdala in 10 rabbits prepared for chronic recording. Four basic taste solutions were delivered to the restrained animals via a multibarreled mouth tube, and responses to these solutions were analyzed. Of 120 amygdala units sampled, 34 were responsive to the tastants. These were localized within, or near, the borders of the central nucleus. Responsive cells were classified as type 1, 2, 3, or 4 depending on the number of tastants to which they responded. Most cells responded to three or more solutions. There were, however, a small number of very selective cells. In all, 25 of the 34 responsive cells could be said to discriminate among tastes. It is likely that this gustatory information arrives at the amygdala via the parabrachial nuclei since the pathway in rat and rabbit are similar. This kind of sensory information could contribute to the amygdala's role in the control of ingestion.

55.8 GOLGI ANALYSIS OF PIRIFORM CORTEX. L. Haberly, Department of Anatomy, University of Wisconsin, Madison, WI 53706

The piriform cortex of the adult opossum (Didelphys virginiana) has been studied with Cox and rapid Golgi techniques.

Most deep pyramidal cells closely resemble those in other parts of the cerebral cortex by virtue of a single apical dendrite, multiple basal dendrites, a large number of small to medium dendritic spines, and a deeply-directed axon. Apical dendrites arborize into tufts of secondary dendrites near the layer I-II border, regardless of depth of the soma. As a result, pyramidal cells in layer II have only a short apical trunk or terminal tufts that emerge directly from the cell body.

With very conservative criteria for categorization, nine different types of non-pyramidal cells have been distinguished. Layer I contains a small number of non-pyramidal neurons with both smooth and spiny dendrites including distinctive fusiform cells with large somatic appendages. The predominant type of non-pyramidal neuron in layer II, as described in other species, is the semilunar cell which has only apically directed dendrites. These apical dendrites can be distinguished from those of pyramidal cells on the basis of a low concentration of dendritic spines that are predominantly large and disc-shaped. A striking feature is the presence of these large spines only on distal dendritic segments. Within layer III, the most frequently encountered non-pyramidal neurons in the present material are multipolar cells with smooth dendrites that resemble the large stellate-basket cells in neocortex. In addition, layer III contains 3 non-pyramidal neuron types with spiny dendrites: a) fusiform and multipolar cells with somatic spines and complex, branched appendages on distal dendrites, b) very large multipolar cells (somas up to 35μm mean diameter) in the ventral part of the cortex with large diameter dendrites that give rise to abruptly-tapering side branches and long filiform spines, and c) multipolar cells with profusely spiny dendrites that closely resemble those of pyramidal cells. In all 3 layers, small neurons with spherical cell bodies and "axoniform" dendrites have been found that resemble the so-called neurogliaform cells that have been described in a variety of brain areas.

A striking feature of the organization of the piriform cortex is that with the exception of the neurogliaform cells, the different types of non-pyramidal neurons tend to be segregated to individual layers or sublayers.

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55.9 OLFACTORY NEOCORTICAL AREAS IN THE CAT. J.L. Price and S.J. Wiegand. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

In addition to the primary olfactory cortical areas which receive direct input from the olfactory bulb, it has recently become apparent that there are olfaction-related areas in the orbital and insular cortex. In order to define these areas in the cat we have recorded physiological responses in the prefrontal and adjacent cortical areas following electrical stimulation of the olfactory bulb. Small injections of wheat germ agglutinin labeled with horseradish peroxidase were then made into the responsive areas to retrogradely label projections from the primary olfactory areas. In other experiments injections of ^{3}H-leucine were made into the largest primary olfactory area, the piriform cortex, to anterogradely label projections to the orbital and insular cortex.

Three cytoarchitectonically distinct areas have been identified which receive direct input from the primary olfactory cortex and which show consistent and relatively short latency responses to olfactory bulb stimulation. The shortest latency responses (15-30 msec) were found in the ventral agranular insular area (AI_V) in the rhinal sulcus immediately adjacent to the piriform cortex. This area was shown to receive a heavy projection from most parts of the piriform cortex, with additional projections from the nucleus of the lateral olfactory tract and the periamygdaloid cortex. Rostral to AI_V slightly longer latency response (25-40 msec) were obtained in the lateral orbital area (LO) and in portions of the ventrolateral orbital area (VLO) on the ventral part of the medial bank of the presylvian sulcus. Both of these areas receive a projection from the anteromedial part of the piriform cortex. There are substantial interconnections between LO and AI_V, and to a lesser extent, between these areas and VLO. AI_V and LO are also interconnected with the medial part of the mediodorsal thalamic nucleus while VLO is interconnected with the submedial nucleus. (See Craig et al., J. Comp. Neurol. 206:28-48, 1982).

Additional, much less prominent olfactory inputs to other areas of the orbital cortex may come from cells deep to the piriform cortex, or from cortico-cortical connections.

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55.10 GUSTATORY CORTEX IN THE RAT DELIMITED BY THALAMOCORTICAL PROJECTIONS, PHYSIOLOGICAL PROPERTIES, AND CYTOARCHITECTURE. R. Norgren, E. Kosar, and H.J. Grill Rockefeller University, New York, N.Y. and University of Pennsylvania, Dept. of Psychology, Philadelphia, Pa.

Despite the numerous studies on the cortical location of taste, a clear delimitation of the boundaries of gustatory cortex in the rat has yet to be presented. Traditionally it has been assumed that gustatory cortex is localized within granular insular cortex as defined by Rose ('28). By using a combination of anatomical and physiological techniques, however, we have been able to demonstrate that taste is represented within the agranular insular cortex subjacent to the granular area. Multi-unit responses were recorded during electrode penetrations positioned parallel to the lateral convexity of the brain. As the microelectode was advanced ventrally in the cortex, a transition in response properties was observed. Tactile receptive fields shifted gradually from the vibrissae, to the lips, into the mouth, and onto the tongue. Tactile responses were then replaced by lingual temperature and subsequently by gustatory responses. Ventral to the gustatory area, neurons responded to olfactory stimuli. Marking lesions were placed at sites of transition in these functional properties, i.e., at the border between regions responsive to tongue tactile, tongue temperature, gustatory and olfactory stimuli. Subsequently, the locations of the marking lesions were reconstructed and found to conform strikingly to cytoarchitectural boundaries. Gustatory cortex in the rat is situated just ventral to the thinning and disappearance of layer IV, within agranular cortex dorsal to the rhinal sulcus. Tongue temperature was found to be represented in the cortical area previously termed gustatory, i.e., in ventral granular cortex as layer IV attenuates. Olfactory responses began at the level of or just ventral to the rhinal sulcus in piriform cortex. Following the electrophysiological localization of gustatory cortex, small injections of 3H-leucine were placed within physiologically defined gustatory thalamus. The resultant labeling of thalamocortical projections was confined to agranular insular cortex and confirmed the location of gustatory cortex as determined electrophysiologically. Control injections placed within thalamic nuclei surrounding VPMpc resulted in labeling situated outside of the agranular insular cortex. This study demonstrates that the thalamic projections and functional properties of the lingual tactile, thermal, and gustatory sensory systems coincide with recognized cytoarchitectural subdivisions of cortex.

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55.11 LACK OF EFFECT OF CHOLECYSTOKININ ON THE INTEGRATED CHORDA TYMPANI RESPONSE. B. A. Gosnell and S. Hsiao. Department of Psychology, University of Arizona, Tucson, Arizona 85721.

Cholecystokinin (CCK) has been reported to reduce sucrose intake in rats maintained on ad lib food and water (Waldbillig and O'Callaghan, Physiol. Behav., 25:25-30, 1980). This decrease may be mediated by a reduction in the hedonic value of the taste. To determine whether this effect is due to a shift in the sensitivity of gustatory receptors, integrated responses were obtained from the uncut chorda tympani nerve.

Male Long-Evans hooded rats were anesthetized with urethane. The right chorda tympani was exposed and placed over two platinum electrodes. Taste stimuli were: concentrations of NaCl (0.01-0.10 M), sucrose (0.05-0.50 M), and Similac infant formula (normal and half-normal dilution). Approximately 0.25 ml of each stimulus was applied to the anterior tongue and left on for approximately 45 sec, after which the tongue was rinsed with distilled water. The interval between stimuli was a minimum of one minute. After each stimulus was applied at least twice, one ml of either CCK-8 (5 µg/ml, Squibb) or NaCl was infused via jugular catheter, and the stimuli were again tested.

No significant changes were found in the amplitude of the integrated response after CCK infusions as compared to NaCl infusions. The dose of CCK (approx. 10-15 µg/kg), however, is higher than that which consistently reduces food and sucrose intake. It is tentatively concluded that the apparent interaction between CCK and orosensory cues is not due to a change at the peripheral sensory level. This is in contrast to the effect of another feeding-related factor, stomach distention, on the peripheral taste nerves of frogs and toads, as reported by Esakov (Bull. Exp. Biol. Med., 51:257-262, 1961) and Brush and Halpern (Physiol. Behav., 5:743-746, 1970). Future work will include measuring the effect of CCK in taste-related areas of greater integrative capacity.

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55.12 EEG-LIKE SIGNALS FROM THE BOVINE VOMERONASAL ORGAN. W.R. Klemm, C.J. Sherry, R.F. Sis*, and D.L. Morris*. Dept. of Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

The vomeronasal organ (VNO) is an accessory olfactory system in the nasal septum that is believed to enhance chemical communication between individuals of the same species; the most generally accepted role is that of allowing males to detect estral females. Other workers have shown that stimulation with certain odorants causes marked increase in the discharge of VNO nerves of the tortoise and the rabbit. We therefore tested the hypothesis that appropriate stimuli would cause generator potentials in VNO sensory cells which collectively would produce recordable field potentials similar to those seen with such sensory systems as the retina or olfactory mucosa. To test the idea, plastic cannulae (10 cm long) were implanted into the VNO ducts of 4 young male castrates under halothane anesthesia. Bilateral incisions (1 cm lateral to the midline, extending 2 cm caudally from the incisive pit) were made through the palatine ridge of the hard palate to expose the junction of the VNO duct and the incisive (nasopalatine) duct. Because the natural topography of the ducts is tortuous, the plastic cannulae were ensheathed in 5 cm metal tubes (12 gauge) which in turn were anchored by sutures to the incisive bone (premaxilla). For recording in the chronic, awake animal, stainless-steel tubing (23 gauge) electrodes were inserted inside the plastic cannulae so that no contact was possible with any skin or mucosa; the sole electrical contact was with the fluids in the VNO and the plastic cannula. Recordings were either between an electrode in the VNO and a subcutaneous reference electrode over the bridge of the nose or bipolar between the two VNO electrodes.

With both kinds of recording, signals were superficially similar to an EEG, exhibiting similar spontaneous amplitude fluctuations. However, many of the large low-frequency components (0.1 to 30 Hz passband) were much slower than found in the typical EEG. Because the recording electrodes double as injection tubes, we are currently investigating the electrical response to perfusion of the VNO with various odorants. Such perfusion commonly elicits licking, chewing, and sniffing movements.

It is not yet clear how much of the signal, particularly during perfusion with chemicals, reflects electrode-potential artifact resulting from fluid motion within the VNO. With that caveat we tentatively propose that these potentials might qualify to be designated, analagously to the ERG and EOG, as the EVNOG, the electro-vomeronasogram.

55.13 CONDITIONED TASTE AVERSIONS MODIFY NEURAL RESPONSES IN RAT NUCLEUS TRACTUS SOLITARIUS. F-C.T.Chang and T.R.Scott. Dep't. Psychology and Institute for Neuroscience, Univ. of Delaware, Newark, DE 19711.

The nucleus tractus solitarius (NTS) has long been suspected of involvement in conditioned taste aversions because of the close anatomical relationship gustatory and vagal afferents have there. We developed a conditioned taste aversion (CTA) to 0.0025 M sodium saccharin (CS) in 12 rats and compared single unit responses from NTS in these animals with those of 12 control rats. Stimuli included the CS plus eleven other salts, acids, sugars and quinine. We found no change in the number of neurons responding to the CS in CTA rats, but a 51% higher mean discharge rate among those which did respond. Post-stimulus time histograms indicated a greater latency-to-peak response from the cells of CTA rats, with the greatest effect occurring for the CS. The across-neuron pattern evoked by the CS had greater similarity with patterns evoked by all other stimuli, implying that the taste quality of saccharin generalized more broadly to those of other chemicals. The largest change in quality occurred for quinine whose neural pattern moved decisively away from those of other non-sweet stimuli and toward those of the CS and sugars. This implies a neural correlate of the rejection response elicited in CTA rats by the now-aversive CS.

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55.14 CHARACTERIZATION OF DISTINCT MORPHOLOGICAL CLASSES OF OLFACTORY RECEPTOR IN TELEOST FISHES, CARASSIUS AURATUS, AND ICTALURUS PUNCTATUS. Jay F. Muller*(Spon: R.E. Marc). Sensory Sciences Ctr. Univ. of TX Graduate School of Biomedical Sciences, Houston, TX.

Three morphologically distinct classes of olfactory receptor and an additional cell type which represents an early stage of senescence for all three, are proposed, based on the results of this study. These are 1) ciliar cell type I, 2) microvillar cell, 3) ciliar cell type II and 4) rod cell. Retrograde transport of horseradish peroxidase by axons in the olfactory nerve to the olfactory organ of both Carassius auratus and Ictalurus punctatus, analyzed with light and electron microscopy provided evidence that there are four types of cells that contain axons in the organs of both species. Olfactory organs of Carassius were treated with papain and dissociated, providing an isolated cell preparation for correlative morphological information. Distribution of the cell types in Carassius was explored with scanning electron microscopy, a preparation that, in addition, offered evidence for rod cell heterogeneity. Rod processes were observed which showed incomplete fusion of cilia or microvilli, recognizable as derived from each of the three receptor types. This, along with their irregular distribution, led to my hypothesis that the rods represent an early stage of senescence, marked by fusion of the receptors' dendritic surface processes. To test the hypothesis, an acid phosphatase-lead stain, designed for ultrastructural analysis, was performed on Carassius olfactory organs, to indicate specific sites of autolytic activity. Such activity was localized in rod processes, as evidenced by the electron dense reaction product, and examples of each receptor type as progenitors were recognized.

Ciliar cells type I are similar to ciliar olfactory receptors found in all vertebrate classes, and microvillar cells are present in olfactory organs of other fishes and in the tetrapod vomeronasal organ. In Carassius and Ictalurus olfactory organs they are both concentrated in approximately one third the area of each lamella, most proximal to the median raphe. Ciliar cells type II predominate in fishes whose nasal sacs have uniform rather than pulsatile water flow. These cells have been described most often as respiratory type or ciliated non-sensory cells. They are morphologically similar to respiratory type epithelium in the nasal cavities of tetrapods and as such, have motile cilia that beat synchronously, indicative of their respiratory role in fishes. In Carassius they occur singly and in aggregates throughout the organ. In Ictalurus they are segregated from the ciliar type I and microvillar cells, in the outer two thirds of the organ. In Carassius and Ictalurus they contain axons that pass through the olfactory nerve into the olfactory bulb, hence they are receptor-neurons as well as respiratory epithelium. New findings introduced here about the ciliar cell type II and the rod cell open a number of comparative and developmental issues for consideration.

55.15 EFFECT OF STIMULUS COMPLEXITY ON THE RESPONSE OF CENTRAL OLFACTORY NEURONS, K. A. Hamilton and B. W. Ache*. Whitney Marine Laboratory, Univ. of Florida, Rt. 1, Box 121, St. Augustine, FL 32084

Recent efforts to understand olfactory integration have concentrated on quality coding achieved by the convergence of primary afferents onto interneurons within glomerular neuropile. Few physiological studies have examined the nature of the output that leaves higher chemosensory processing centers. Multimodal interneurons descending the circumesophageal connectives from the brain to the lower nervous system in decapod crustaceans respond to stimulation of cephalic sensory structures. Using intracellular recording and staining and backfilling techniques, we have examined the dendritic branching pattern and the effect of stimulus complexity on activation of excitatory descending interneurons during antennular (olfactory) stimulation in the spiny lobster.

Variation in the response of excitatory interneurons is attributable to differences between chemostimulants (ANOVA; n = 31). Two natural mixtures of amino acids plus betaine at concentrations found in potential foods ("crab" and "urchin") are more excitatory than some single components (e.g., glutamate). Other single components (e.g., taurine), however, are as excitatory as equimolar natural mixtures. Most interneurons individually are excited by single components (e.g., taurine and glutamate) that stimulate different primary receptors (Fuzessery, Z. M., et al., Biol. Bull., 154: 226-240, 1978). Response patterns for single components resemble those for mixtures.

Several morphological types of excitatory interneurons can be identified on the basis of dendritic branching pattern. One characteristic that many chemoexcitatory interneurons have in common is extensive branching within the ipsilateral antennal neuropile. These neurons do not appear to contact the olfactory lobes, and thus they presumably are at least third-order chemosensory fibers.

In other crustaceans, high-order descending interneurons like these encode visual information based on stimulus-dependent impulse coordination within an intracting array, rather than in parallel lines (Wood, H. L. and Glantz, R. M., J. Neurophysiol., 43: 741-753, 1980). Because it is unlikely that chemosensory information is further processed per se in lower motor centers which are the targets of these descending cells (Ache, B. W. and Sandeman, D. C., J. Comp. Physiol. A, 140: 295-301, 1980), our results are consistent with the idea that the descending chemosensory "message" also is encoded by interneurons working ensemble.

56.1 BIMODALITY OF AXON SIZE IN THE CAT OPTIC NERVE: A QUANTITATIVE ELECTRON MICROSCOPIC ANALYSIS.

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Previous studies on the size of fibers in the mammalian optic nerve and tract have reported a unimodal diameter spectrum. In view of the strong evidence for discrete conduction velocity groups, particularly in the cat, this unimodal distribution is puzzling. We now report an unequivocal bimodal distribution of fiber size in the adult cat optic nerve. Cats were perfused with mixed aldehydes. The nerves were dissected free and processed for routine electron micrography. A set of micrographs of each ultrathin section was taken at x5000. These micrographs provided a uniform and unbiased sample of the axon population. The following measurements of fiber dimension were made using a Zeiss Videoplan image analysis system: i. internal axon perimeter, ii. area of the axoplasm, iii. diameter of a circle with an equivalent area, iv. fiber diameter (axon and myelin sheath), and v. myelin sheath thickness. Because axon packing density is not uniform throughout the nerve we were particularly careful to test for regional variation in axon size. A peripheral crescent of each nerve had exceptionally high axon packing density. It was found that this peripheral crescent had a relative abundance of smaller caliber axons. Conversely, regions of low density contained a disproportionate number of larger fibers.

In two normal cats a clear bimodal distribution of axon diameter was evident in virtually all regions of both nerves. The total axon population from each nerve also showed a striking size bimodality (n of axons: 12,574 and 11, 936). About 45% of the axons fell under the first peak, 50% under the second peak, and the remaining 5% comprise the extensive tail of the histograms. The first mode of the axon diameter distribution is at 0.8 μm, the second mode is at 2.4 μm. The trough in the spectrum falls at 1.5 μm. The ratio of internal axon diameter to outside fiber diameter was 0.75, and did not vary with axon size. Thus the myelin sheath thickness is a linear function of axon diameter. An analysis of axon diameter in an adult cat which had one eye removed more than two weeks before birth (embryonic day 45) also showed clear bimodality (n of axons: 14,100). Preliminary study of axon size spectrum in an embryonic day 48 cat shows a unimodal diameter distribution with a broad peak at 0.35μm.

Our anatomical results are concordant with data on the velocity of impulse conduction of single fibers in the optic nerve and tract of the adult cat.

56.2 LOSS OF AXONS IN THE CAT OPTIC NERVE: EFFECTS OF PRENATAL UNILATERAL ENUCLEATION.

Robert W. Williams, Michael J. Bastiani, and Leo M. Chalupa. Department of Psychology, University of California, Davis CA 95616 and Department of Biological Sciences, Stanford University, Stanford CA 94305.

We have previously shown that retinal projections are more widespread in fetal than in adult cats. In the present study we asked whether this early and more extensive projection could, in part, result from an excessive number of retinal efferents. Direct estimates of axon number were made from transverse ultrathin sections of the optic nerve. Five nerves have been examined; one taken from a 48 day fetus, two from normal adults, and two from cats which had one eye removed more than two weeks before birth (prior to the 48th day of gestation). Cats were perfused with mixed aldehydes, and following dissection the nerves were prepared for routine electron microscopy. A set of micrographs of each section was taken with a Hitachi HU-11E electron microscope at x5,000 to x25,000. These micrographs provided a uniform and unbiased sample of axon packing density across the entire face of the nerve. The area of the section was measured from a complete low power EM montage taken with a Zeiss EM109.

On embryonic day 48 the optic nerve contained 328,000 ± 17,500 axons (n of micrographs: 167). This estimate of axon number is more than twice that calculated for the two normal adults: 159,000 ± 3,400 and 158,000 ± 4,000 (n of micrographs: 129 and 139). Thus a massive attrition of the ganglion cell population may, in part, underlie the reformation of retinofugal projections which occurs before birth.

We next enquired to what extent prenatal binocular competition contributes to this normal ganglion cell loss. Since prenatal eye removal potentially doubles the postsynaptic target volume available to the spared eye (cf. Rakic, 1979), one might anticipate a total arrest of ganglion cell loss. The two unilateral enucleates had axon counts of 200,000 ± 4,700 and 198,000 ± 4,800 (n of micrographs: 228 and 119). Thus the abolishment of competition between the eyes reduces the cell death incidence from about 50% to less than 40%. This change results in a final neuronal population 25% above normal. These data indicate that although prenatal binocular competition is a significant factor governing the severity of normal ganglion cell loss, other mechanisms must also play a role in establishing the final ganglion cell population size.

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56.3 CONTRALATERAL AND IPSILATERAL RETINAL PROJECTIONS IN JUVENILE AND ADULT CHANNEL CATFISH, ICTALURUS PUNCTATUS. P.D. Prasada Rao* and S.C. Sharma. Department of Ophthalmology, New York Medical College, Valhalla, NY 10595.

The retinal projections of the juvenile and adult channel catfish, Ictalurus punctatus, were studied using Horseradish peroxidase (HRP) and radioautographic techniques. The optic fibers do not decussate completely at the level of the optic chiasm and a few project to the diencephalic nuclei and tectum ipsilateral to the retina. A majority of the retinal fibers extend to the contralateral side to form the optic tract, a few of which peel off and project to the suprachiasmatic nucleus (SCN) located ventrolateral to the preoptic recess. The optic tract divides into lateral (LOT) and medial optic tracts (MOT). In the adult fish the LOT is thicker than the MOT, whereas in the juvenile form the reverse is true. The MOT curves laterally and divides into 8-15 medial fascicles of the optic tract (MFOT). The presence of several MFOT might be associated with the multiple optic papillae known to exist in this species. A few fibers of the MFOT and LOT join to form the dorsomedial fascicle of the optic tract (DMFOT) which innervates the nucleus opticus dorsolateralis (NODL) and extends posterodorsally to issue fibers to the nucleus of the posterior commissure (NCP). Subsequently, a few fibers extend further caudal to the posterior commissure, curve laterally and traverse the torus semicircularis to project to the deeper layers of the tectum. The LOT also receives some fibers from the MFOT and projects to the nucleus geniculatus lateralis, pretectal nuclear complex and nucleus corticalis. The stratum fibrosum et griseum superficiale of the tectum is profusely innervated, while the stratum griseum centrale also receives a few retinal fibers. The tractus opticus accessorius arises from the inner margin of the posterodorsal region of the LOT and extends ventromedially to project to the nucleus opticus accessorius (NOA).

The undecussated retinal fibers project to almost all ipsilateral sites similar to those of the contralateral side including the tectum. However, the ipsilateral retinal fibers are mostly located in the anterior part of the tectum indicating that this is the region associated with binocular vision in the catfish. The autoradiographic observations substantiated the analysis of retinal fiber projections based on HRP technique particularly regarding the terminal sites. Since some previous studies also demonstrated the presence of ipsilateral retinal fibers in relatively few aberrant species of teleosts, it is argued that their existence might be an adaptive feature depending upon the environment and the animal's visual capacity having no bearing on the evolutionary status.

56.4 EVIDENCE FOR A RETINAL PROJECTION TO THE SUBSTANTIA NIGRA IN THE RAT, CAT AND MONKEY. A. Frankfurter and R.M. Beckstead. Depts. of Neurological Surgery and Anatomy, University of Virginia School of Medicine, Charlottesville, VA 22908

Fifty years ago, Le Gros Clark described a retinal projection to the substantia nigra in the rat based on Marchi-stained tissue. Gillilan (J.C.N.,1941,74:367-408) using the same method confirmed his finding in a variety of animals,including the cat and monkey. However,Hayhow et.al.(J.C.N.,1960,115:187-216) using the Nauta technique were not able to replicate these earlier studies,and following their report there has been no further discussion in the literature regarding a retinonigral projection.

We are currently re-examining the central distribution of retinal axons in the rat,cat and monkey using horseradish peroxidase as an anterograde tracer.In these animals,we have been able to consistently identify labeled retinal axons in the contralateral substantia nigra,pars reticulata following intravitreal injections.These axons are of extremely fine caliber,and can best be identified by viewing tetramethyl benzidine reacted tissue with darkfield optics through cross-polarizing filters.

Retinal axons enter the substantia nigra by three routes.Some fibers pass through the medial terminal nucleus before sweeping ventrolaterally,while others either perforate the subjacent cerebral peduncle or course around its dorsolateral border.Within the substantia nigra,labeled axonal fragments appear to take a trajectory parallel to the mediolateral axis of the nucleus.These fragments are not characteristic of fibers of passage;some give off sidebranches,while others undulate through the nucleus possibly making en passant pericellular contacts.The distribution of these axons within the substantia nigra is particularly intriguing:in all three species,the majority of labeled axons are located in the rostral half of the nucleus,and their distribution within this segment is roughly coincident with the location of pars reticulata neurons which project to the superior colliculus.For example,in the monkey most of the axons enter the dorsolateral segment of pars reticulata (Beckstead and Frankfurter,J.Neurosci. 1981,1:121-125).Since it has been demonstrated that nigrotectal cells decrease their firing rate following visual stimulation (Wurtz and Hikosaka,Neurosci.Abs.,1981,7:132),it is tempting to propose that the function of this pathway is to inhibit nigrotectal neurons through an interneuronal relay.In addition,our observation of sizable numbers of retinal axons within the substantia nigra may have important implications for assessing the behavioral consequences of nigral lesions.

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56.5 AFFERENT AND EFFERENT CONNECTIONS OF THE ACCESSORY OPTIC SYSTEM IN THE CAT, M.T. Feran* and K.L. Grasse (SPON: G. Mandl). Dept of Psychol., Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4J1.

Recent investigations have demonstrated projections to the inferior olivary complex (IOC) from midbrain sites including the pretectum, oculomotor complex and superior colliculus. Anatomical studies in pigeon and rabbit have revealed an additional fiber projection arising from the accessory optic system (AOS). Although the afferent input to the cat AOS has received some attention, very little is known about the efferent projections of the AOS in this animal.

We have examined the input and output fiber systems of the cat AOS using autoradiographic and HRP tracer techniques. Following microinjections of HRP (0.4 µl of 30% Sigma VI in saline) primarily into the rostral IOC, labelled cells were observed in the periaquaductal gray (PG), the nucleus of Darkschwitch (ND), the interstitial nucleus of Cajal (INC), the posterior pretectal nucleus (PPN), and the nucleus of the optic tract (NOT). When HRP injections were centered more caudally in the IOC, labelling was observed in the PG, the ND, the PPN, and in the dorsal aspect of the medial terminal nucleus (MTN) of the AOS.

Intravitreal eye injections of ^{3} H-proline (2 mi. cu. in 35 µl saline) revealed the dorsal, lateral and medial terminal nuclei (DTN, LTN, MTN) of the AOS. Input fibers of the accessory optic tract (AOT) synapse first in the DTN after leaving the brachium of the superior colliculus, then proceed ventro-medially to the LTN along the base of the midbrain. After leaving the LTN the fibers of the AOT sub-divide into at least two sparse networks of axons: 1 set of fibers span out and penetrate the substantia nigra and cerebral peduncle before terminating in the MTN, while another group of fibers course along the base of the midbrain en route to the MTN. Both sets of input fibers terminate primarily in the ventral MTN. In contrast, the HRP-filled MTN cells follow -ing IOC injections are more heavily concentrated in the dorsal aspect of the MTN.

Thus, there is apparently an internal organization within the cat MTN: while the afferent fiber system from the retina primarily contacts cells in the ventral MTN, the efferent fiber system originates in cells more heavily concentrated in the dorsal region of the MTN.

56.6 DESCENDING PROJECTIONS OF THE MEDIAL TERMINAL NUCLEUS OF THE ACCESSORY OPTIC SYSTEM: A LIGHT AUTORADIOGRAPHIC STUDY IN RAT AND RABBIT. Robert H.I. Blanks[1]; Roland A. Giolli[2]; Yasuhiro Torigoe[3]* Dept. Anatomy[1-3] & Surgery[1], U. Calif. Irvine, Irvine, CA 92717

The medial terminal nucleus (MTN) receives direct retinofugal projections, contains neurons which are speed- and direction-selective for vertically moving visual targets, and on the basis of anatomical and lesion studies, mainly in birds, is thought to be involved with some aspect of visuo-motor control (e.g., optokinetic nystagmus). However, the efferent projections of this nucleus are poorly understood in mammals. The present study examined projections from MTN which had been injected with ^{3}H-leucine in 13 rats and 20 rabbits.

The data from both species revealed remarkably similar results. There are 5 major bundles arising from the MTN to terminate within the midbrain and brainstem. The largest bundle, described previously (Blanks et al., 1982), terminates within the ipsilateral pretectum. The second bundle courses through and around the red nucleus with ipsilateral projections to the interstitial nucleus of Cajal (INC). From this same bundle, axons ascend dorsally through the posterior commissure and then descend to terminate in the contralateral INC, ventral tegmental nucleus of Tsai (VTA) and red nucleus (parvicellular). The third bundle courses medially, ascends the midline to enter the medial longitudinal fasciculus (MLF) and courses within the ventromedial and medial portion of MLF to the posterior medulla. The axons then descend along the midline and terminate within the rostral part of the dorsal cap of the inferior olive. The fourth bundle ascends laterally to the medial lemniscus, turns caudally and distributes many terminals to the ipsilateral midbrain reticular formation, parabrachial and dorsal pontine reticular formation with a smaller bundle distributed to the periaqueductal grey at the level of the oculomotor complex. The fifth bundle courses medially through VTA to the contralateral side, courses posteriorly and dorsally to cerebral peduncle, follows lateral lemniscus, finally at the level of the mesencephalic trigeminal nucleus, the bundle assumes a position between cerebellar white matter and brainstem where it terminates on the large neurons of the superior vestibular nucleus, with some axons continuing caudally to terminate more posteriorly within the vestibular complex. There were no direct projections to the oculomotor nuclei.

These results demonstrate that the MTN has a number of important connections to midbrain and brainstem structures known from physiological studies to be involved with vertical eye movements. These findings, combined with the receptive field properties of MTN neurons, support the general conclusion that this nucleus plays an important role in controlling vertical eye movements.
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56.7 DEVELOPMENTAL CHANGES IN PROPERTIES OF SINGLE UNITS IN THE AVIAN ACCESSORY OPTIC SYSTEM, Sheila Burns and Josh Wallman. City College of City Univ. of N.Y., New York, N.Y. 10031.

The accessory optic system has an essential role in stabilizing eye movements. In mature chickens, single units in the nucleus of the basal optic root (nBOR) respond to slowly moving directional stimuli and form two homogeneous and well-defined classes: one which is excited by upward movement and inhibited by downward movement and a second which is excited by movement downward and anterior and inhibited by movement upward and anterior. These two classes of cells are found in separate parts of nBOR; units which respond to upward movement are located in the dorsal part, while units which respond to downward movement are located in the ventral part.

There is anatomical and behavioral evidence that a different pattern exists in young chicks. 2-deoxyglucose studies show that the dorsal and ventral segregation of upward and downward units demonstrable in older chickens is not yet present in young chicks. In addition, measurements of optokinetic stabilizing eye movements show that while mature chickens respond best to movements which are downward and clockwise, young chicks show the greatest gain to movements which are upward and clockwise. These two lines of evidence indicate that a developmental reorganization of nBOR is occurring.

We have begun to study single units in nBOR in chicks less than one week old. Preliminary evidence indicates that there are units that do not fit into the classes described above and that at least some of the units are not sharply directionally tuned. Detailed information on the directional tuning curves of these units and their anatomical location within nBOR will be presented.
(Supported by NIH EY2937)

56.8

WITHDRAWN

56.9 THE ANURAN NUCLEUS PRETECTALIS (LARGE-CELLED NUCLEUS): NEUROANATOMICAL AND FUNCTIONAL ANALYSIS. Neil Montgomery*, Antony M. Grigonis*, Katherine Fite, Lynn Bengston*, University of Massachusetts, Amherst, Massachusetts, 01003.

Recent studies have demonstrated that the pretectal region is critically involved in mediating horizontal optokinetic nystagmus (OKN). Lesions of either n. pretectalis (nPt) or the pretectal tegmental gray appear to have equivalent effects in reducing OKN.

The pretectal region was investigated using autoradiographic (^{3}H proline), horseradish peroxidase and Golgi techniques. Retinal afferents to nPt originate from the central portion of the retina and project via the axial optic and marginal optic tracts. The primary projection is contralateral with a small ipsilateral component. Fibers related to the axial optic tract arborize first in a relatively cell-free "dense-core" region and then branch throughout the surrounding region. Other afferents to nPt originate from the ipsilateral tectum, the nucleus of the basal optic root, the nucleus of the posterior commissure and the anterior thalamic nucleus rotundus. Three types of neurons occur in nPt; large neurons (25 µm), fusiform neurons (12.5 µm), and stellate neurons (10 µm). Additionally, two cell groups outside of nPt send dendrites into the nucleus; cells in the posterior lateral nucleus and cells in the central gray dorsal to the central thalamic nucleus. Both the large neurons and the fusiform neurons of nPt project to the anterior and caudo-lateral portions of the tectum as well as to the ventral brainstem superficial to the abducens nucleus (n. VI). The stellate neurons appear to be intrinsic to nPt. Cells in the central gray postsynaptic to nPt project directly to n. VI.

The anuran nPt appears similar and perhaps homologous to the nucleus of the optic tract of mammals both in terms of its presumed role in horizontal OKN (Hoffman and Schoppmann, 75), the site of origin of its retinal afferents (Ballas et al., 81) and its central connections (Berman, 77).

56.10 EFFERENT PROJECTIONS OF THE PRETECTAL COMPLEX: DIFFERENT POPULATIONS OF NEURONS PROJECT TO LATERAL THALAMUS AND TO INFERIOR OLIVE OF THE RAT. Richard T. Robertson and Janie L. Callaway*. Department of Anatomy, College of Medicine, University of California, Irvine, CA. 92717.

The pretectal complex consists of several nuclear groups, including the anterior (PA), posterior (PP), medial (PM), olivary (PO) and optic tract (TO) nuclei. Several recent studies have demonstrated both descending projections to the inferior olive complex and ascending projections to the lateral thalamic group. The present studies used the double retrograde labeling technique with two fluorescent markers to determine whether the two projections are axon collaterals from one population of neurons.

Adult Long-Evans hooded rats received injections of either nuclear yellow (5% in water) or a mixture of true blue and granular blue (5% in water) in either the thalamic lateral dorsal nucleus (LD) or the inferior olive complex (IO). After survival periods of 20-48 hrs, animals were sacrificed by aldehyde perfusion and 30 µm serial frozen sections were examined by fluorescence microscopy. Nuclear yellow labels the neuron nucleus yellow; granular and true blue label the cytoplasm blue. Thus, double labeled cells are easily discerned.

Injections in LD resulted in retrogradely labeled neurons in all pretectal nuclei. Most prominent labeling occurred in PO, PA, and PP. Injections in IO resulted in retrogradely labeled neurons in PP, TO, and PA. In fifteen experiments, we have examined more than 2000 labeled neurons. Each of these neurons was labeled by only one compound; in no case have we observed double labeled neurons in the pretectal complex. This lack of double labeled cells appears not to be an artifact of our methodology; in other experiments with different injection sites we commonly have seen double labeled neurons.

These data indicate that the projections to IO and LD originate in separate populations of neurons of the pretectal complex. These results suggest that extra-geniculate visual information destined for either cerebellar or cerebral cortex passes through separate neuronal channels in the pretectal complex.

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56.11 SUPERIOR COLLICULUS: INTERACTIONS OF CONVERGING MULTISENSORY INPUTS, M. A. Meredith and B. E. Stein, Dept. of Physiology, Medical College of Virginia, Richmond, VA 23298.

Visual, auditory, and somatic cells are located in the intermediate and deep laminae of the superior colliculus (SC). Although many of these neurons are influenced by more than one sensory modality (Stein, B. E. and Arigbede, M. O., Exp. Neurol. 36:179, 1972), the manner in which multiple inputs interact to determine cellular activity is not known. The present experiments were initiated to investigate such interactions in order to gain insight into the functional role of multimodal convergence.

Experiments were conducted using cats (n=4) and hamsters (n=3) which were paralyzed with gallamine triethiode and artificially respired with N_2O and O_2, as well as hamsters (n=16) anesthesized with urethane. Visual stimuli consisted of stationary and moving spots and bars of light. Auditory stimuli were hisses delivered by a solenoid-controlled air hose or clicks from an audio-amplifier. Tactile stimuli were delivered by a solenoid-controlled air stream or with an electronically-driven moving coil vibrator. Once a cell was isolated (n=66) its responses to separate visual, auditory and tactile stimuli were tested and optimal stimuli were determined. Combinations of stimuli from these different modalities were then presented simultaneously and at various predetermined intervals.

Despite the fact that "optimal" stimuli in each modality were identified, responses evoked by these separately presented stimuli were often not maximal. For example, cells excited by two or more modalities (n=26) showed facilitory interactions when stimuli were delivered in close temporal proximity to one another. On the other hand, cells were encountered which were excited by one input and inhibited by another (n=15), and suppressive interactions were exhibited by these units. In general, most interactions were generated by paired stimuli delivered within ±100 msecs of one another. Although no cross-modality interactions were seen in cells of the superficial laminae, such interactions were observed in the majority of cells in the intermediate and deep layers.

These data indicate that information from different modalities is integrated in SC neurons through facilitory and/or suppressive interactions. When the behavioral role of the SC is considered, these interactions may serve to enhance the detection and localization of stimuli.

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56.12 ULTRASTRUCTURAL CHARACTERIZATION OF THE INTERCOLLICULAR PATHWAY IN THE CAT. Mary Behan, Department of Structural and Functional Sciences, University of Wisconsin School of Veterinary Medicine, Madison, Wisconsin 53706.

Following ablation of the occipitotemporal cortex in the cat, a contralateral hemianopsia results which can be reversed by section of the intertectal commissure or by destruction of the contralateral superior colliculus (Sprague, J.M., 1966, Sci. 153: 1544). To explain this phenomenon, it has been suggested that the superior colliculi functionally suppress each other, and that this effect is mediated by the intertectal commissure. This projection connects the stratum griseum intermediate of the rostral half of the colliculi, and many fibers connect corresponding points in the colliculi (Edwards, S.B., 1977. J.Comp.Neur. 173:23).

In this study, EM-autoradiography has been used to investigate the synaptic connections of the tecto-tectal pathway. In two cats, two injections (0.2-0.3 µl) of ^{3}H-proline or a mixture of ^{3}H-proline and ^{3}H-leucine (50 µCi/µl) were made in the rostral half of the left colliculus. Following a 24-30 hour survival, the animals were perfused and the superior colliculi embedded for electron microscopy. Thin sections were cut from the rostral half of the right colliculus in an area corresponding to the injection sites. Only the stratum griseum intermediale was included in the sections which were subsequently processed for EM-autoradiography.

Based upon vesicle shape, two populations of synaptic terminals are labeled. Type I contains round synaptic vesicles and comprises 62% of all labeled terminals. Most of these terminals make asymmetric contacts upon dendrites, although a few synapse upon dendritic spines. It appears that each type I terminal makes only a single synaptic contact. Synaptic vesicles are scattered unevenly in 79% of the type I terminals, but frequently are clustered at the synaptic contact. The remaining 21% of type I terminals contain densely packed round synaptic vesicles.

Type II terminals, comprising 38% of those labeled, contain pleomorphic or flattened vesicles and make symmetric contacts upon dendrites. Each type II terminal appears to make only a single synaptic contact. Synaptic vesicles are unevenly distributed in approximately 93% of the type II terminals examined, and in some terminals there is clustering of vesicles at the synaptic contact. Preliminary measurements indicate that there is substantial overlap in the size distribution of the two populations of terminals.

While it is certain that many of these labeled profiles are indeed terminals of intercollicular neurons, the possibility exists that some are associated with collaterals of other decussating fiber systems, e.g., the tecto-parabigeminal and tecto-cuneiform projections.

56.13 DISTRIBUTIONS OF CHOLINE ACETYLTRANSFERASE AND ACETYLCHOLINESTERASE ACTIVITIES IN LAYERS OF RAT SUPERIOR COLLICULUS. C.D. Ross, J.L. Park*, K.G. Ledbetter* and D.A. Godfrey, Dept. Physiology, Oral Roberts University, Tulsa, OK 74171

The rat superior colliculus is divided into 7 layers. Superficial layers include (1) stratum (s.) zonale, (2) s. griseum superficiale, (3) s. opticum; deep layers include, (4) s. griseum medium, (5) s. album medium, (6) s. griseum profundum, (7) s. album profundum. Optic nerve fibers proceed through layer 3 and terminate primarily in layers 1 and 2 (Lund, J. Comp. Neurol. 135: 179, 1969). Non-optic pathways terminate in the deep layers.

Activities of the enzymes of acetylcholine metabolism, choline acetyltransferase (ChAT) for synthesis and acetylcholinesterase (AChE) for degradation, were assayed in samples of superior colliculus layers dissected from 20 µm-thick freeze-dried sections cut in the sagittal plane. The average sample size was about 0.15 mm by 0.5 mm in the dorsal-ventral and caudal-rostral orientations, respectively. High AChE activity (150-200 mmoles/kg dry wt/min, 37°) was found in superficial layers 1 and 2, lower activity (30-70) in layers 3, 5, 6 and 7, and intermediate activity in layer 4 (average 80-100). AChE activity in layer 4 was highest caudally (150) and gradually declined to about 30 in the most rostral part of the colliculus. ChAT activity was low to intermediate in all layers (200-500 µmoles/kg dry wt/min, 37°) except in layer 4 (average 600-800). Like AChE, the ChAT activity in layer 4 was highest most caudally (1300) and gradually declined to 200-300 in the most rostral part of the colliculus. (For comparison, average activities for rat whole brain are about 45 mmoles/kg dry wt/min for AChE and 500 µmoles/kg dry wt/min for ChAT.) The relative distributions of both enzymes in the colliculus are consistent with each other except in the superficial layers, where the AChE activity is much higher than would be expected from the activity of the more definitive marker for cholinergic structures, ChAT. The activities in layer 4 could be related to a proposed cholinergic pathway from the substantia nigra terminating in this layer predominantly in the more caudal aspect of the colliculus. A patchy appearance of the AChE stain in the deep collicular layers in cat, monkey and human has suggested a columnar organization that may coincide with the termination of the nigrotectal tract (Graybiel, Neuroscience 4:643, 1979). We are investigating whether the quantitative distributions of ChAT and AChE activities correlate with this appearance of the AChE stain in rat.

In a separate experiment, one week after enucleation of one eye, there were no differences in either ChAT or AChE activities in collicular layers between lesion and control sides.

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56.14 EFFECTS OF PICROTOXIN AND BICUCULLINE ON VISUAL RESPONSE OF ZEBRAFISH TECTAL CELLS. P. Sajovic and C. Levinthal, Dept. Biological Sciences, Columbia University, New York City, N.Y. 10027

In previous work we described four types of visual response among tectal cells of the zebrafish. Cells of one class, type I, are not spontaneously active and respond phasically at ON and OFF. Their responses to moving edges, to growing stimuli, to stimuli equal in size and shape to the whole RF, to pairs of spots, and to very small stimuli all suggest that these cells receive inhibitory input from near neighbor cells of the same type in the tectum, as well as excitatory input from retinal fibers (Sajovic and Levinthal, Neuroscience, in press). In order to investigate this hypothesis further we have studied the effects of drugs on physiological properties of type I cells recorded in the SPV layer of the zebrafish tectum.

Small (10-100 nL) injections of drugs were made in the tectum while recording 100-500 microns away with extracellular microelectrodes. Both picrotoxin (saturated solution in 100mN HCl) and bicuculline (5 mM in 165mM NaCl) produce the following effects: (1) Onset of spontaneous bursting multiunit activity. This noise can be recorded at all depths within the tectum. (2) Abolition of the second postsynaptic wave of the field potential elicited in the tectum as the result of a shock applied to the optic nerve. This wave, which is opposite in polarity to the first postsynaptic wave and follows it by 4 ms, can be interpreted as the product of massed inhibitory postsynaptic currents evoked with a delay after the excitatory currents that give rise to the first postsynaptic wave. It is abolished, while the peak value and time to peak of the first wave are unchanged. (3) Alteration of visual response properties of individual type I tectal cells. Some cells which are originally not spontaneously active emit spontaneous bursts after the drug takes effect; others do not. The duration of response to small flashing spots and to stimuli that grow in size both increase significantly. Responses to moving edges, which normally occur mostly as the edge is crossing the RF border, become extended to encompass the entire RF. Finally, the cells show reduced negative spatial summation following drug injection.

All of these effects are fully reversible with time after injection. Bicuculline effects wash out much faster than picrotoxin (about 10 min vs 2 hr). Control injections (of teleost Ringer's solution, 100 mN HCl, and 165mM NaCl) do not elicit any of these effects. The results reported here are consistent with the hypothesis that tectal type I cells receive a delayed inhibitory input, probably via GABA synapses, which determines major properties of the visual response.

56.15 LAMINAR ORIGIN AND CYTOARCHITECTURE OF SUPERIOR COLLICULUS CELLS PROJECTING TO SUBCORTICAL VISUAL SYSTEM STRUCTURES. N. Lugo-García and E. Kicliter, Lab. Neurobiology and Dept. of Anatomy, University of Puerto Rico, Sch. of Med., San Juan, P.R. 00901.

The superficial laminae of the superior colliculus (SC) in the ground squirrel Spermophilus tridecemlineatus are known to project to several visual system nuclei. Among these are the ventral lateral geniculate nucleus (LG_V), pretectum (PT), the parabigeminal nucleus (Pb) and nucleus lateralis posterior (LP). We were interested in comparing the laminar location and cytoarchitecture of collicular cells projecting to each of these structures. Horseradish peroxidase (HRP) was iontophoretically injected into LG_V, PT, Pb and each of the three subdivisions of nucleus lateralis posterior: rostrolateral, rostromedial and caudal LP. After survival periods of 24-48 hours, the animals were perfused intracardially and brain sections processed according to the method of de Olmos and Heimer (1977). In all of the cases labeled cell bodies were located in the superficial laminae of the superior colliculus. After injections into LG_V, PT and Pb, HRP-filled neurons were present in the upper and lower halves of stratum griseum superficiale (SGS) and in the upper portion of stratum opticum (SO). The labeled cells were mainly multipolars with radiating dendrites or fusiform neurons with cell bodies oriented perpendicular to the tectal surface. Some differences were found in the exact location and soma size of the neurons projecting to each of the injected nuclei. Cells labeled after injections into nucleus lateralis posterior were large multipolar neurons specifically located in the lower half of stratum griseum superficiale. These cells were generally larger than those projecting to LG_V, PT and Pb. While only the ipsilateral SC was labeled after injections into rostrolateral LP, after injecting caudal LP labeled cells were also found in the contralateral superior colliculus. No labeling was present in SC after injections into rostromedial LP. These findings indicate a segregation in the superior collicular cells projecting to other visual system structures.

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56.16 A POSSIBLE SECOND ASCENDING AVIAN TECTOFUGAL PATHWAY. P.D.R. Gamlin and D.H. Cohen. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

Visually conditioned heart rate change in the pigeon has been developed as a vertebrate model for cellular studies of learning. In this system we have determined that the thalamofugal and tectofugal ascending visual pathways participate in transmitting the conditioned stimulus information. Previous results also suggest the involvement of a third ascending visual pathway, possibly of pretectal origin. However, the lesions on which this hypothesis is based also encroached upon the nucleus dorsolateralis posterior of the thalamus (DLP), a nucleus that has recently been reported to receive a tectal projection. This motivated the present investigation of whether there exists a retino-tecto-DLP-telencephalic visual pathway.

Horseradish peroxidase (HRP) injections in DLP labelled cells in a number of regions, including the optic tectum. Labelled tectal cells were present mainly in layer 13 (the stratum griseum centrale), although a few labelled neurons were also evident in layers 8-10 and 14-15.

Injection of ^{3}H-proline/leucine in DLP indicated that DLP projects to a discrete region of the neostriatum intermedium (NI). The terminal field is located immediately dorsal to the lamina medullaris dorsalis and medial to the caudal aspect of the ectostriatum. It extends from approximately A7.25 to A9.0 in the atlas of Karten and Hodos (1967). A less prominent projection to the hyperstriatum was also observed.

HRP injected into this neostriatal terminal field labelled numerous cells in the posterior region of DLP, extending from A4.5 to A5.25. This confirmed that at least part of DLP projects to NI, and it suggests that the anterior region of DLP may project to the hyperstriatum.

Single unit activity of cells in DLP indicated that 13 of 14 neurons studied were responsive to whole-field illumination of the retina. The latency range was 30-55 msec. Eleven of these neurons responded with excitatory bursts at stimulus onset and termination.

These findings suggest that most cells in DLP are visually responsive with latencies comparable to those of neurons in the nucleus rotundus, the thalamic relay of the tectofugal pathway. The results further suggest a retino-tecto-DLP-neostriatal pathway that transmits visual information to the telencephalon. This would constitute a second tectofugal visual pathway to the telencephalon that parallels the well-described retino-tecto-rotundo-ectostriatal pathway. (Supported by NSF grant BNS 8016396 to to DHC.)

56.17 EFFECTS OF LESIONS OF THE CORE NUCLEUS ON VISUAL INTENSITY DIFFERENCE THRESHOLDS IN TURTLES. W. E. Grisham* and A. S. Powers. Department of Psychology, Bryn Mawr College, Bryn Mawr PA 19010.

Preoperative intensity difference thresholds were assessed in eastern painted turtles (Chrysemys picta picta). The method used was to train the animals to discriminate between a very bright and a very dim stimulus. The stimuli were presented on two keys in a standard turtle chamber by a Kodak Carousel projector. The animals were required to make a simultaneous discrimination. After reaching criterion on the training pair (.1 vs. 2.0 log units of optical density) the turtles were trained on a procedure in which 28 trials were given per day, eight with the training discriminanda and four with each of five more difficult discriminations. The animals were always reinforced (with beef baby food) for responding to the brighter stimulus (.1 log units of optical density). Since relatively little data could be collected per day, each threshold determination used data from four consecutive days. To determine the threshold, performance on each of the problems was plotted, and the threshold was taken as the point where the turtle responded correctly 75% of the time (50% being chance). Preoperative criterion was reached when the threshold did not vary more than .04 log units of optical density in two consecutive four-day blocks.

Following achievement of preoperative criterion, each animal was given bilateral electrolytic lesions aimed at the core nucleus (CN) of the dorsal ventricular ridge, the telencephalic termination of the tectofugal visual pathway. Postoperatively, there was no permanent elevation of threshold, even in those subjects with the largest amount of damage to the CN. Subjects with moderate to severe lesions, however, required further practice on the training pair before beginning threshold assessment postoperatively. In addition, there was increased variability of performance in all CN-lesioned subjects: all required substantially more training postoperatively than a control subject with no damage to the dorsal ventricular ridge. The results are consistent with other findings from our laboratory that indicated that turtles with CN lesions had a postoperative deficit on visual intensity discriminations, but were able eventually to relearn them. The present findings contrast with those found after lesions of nucleus rotundus thalami, which projects to the CN. Severe lesions of nucleus rotundus produced permanent elevations in visual intensity difference thresholds.

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56.18 THE INFLUENCE OF RETINO-COLLICULAR PATHWAY STIMULATION ON LATERAL GENICULATE CELL RESPONSES. D. Delaunais*, S. Molotchnikoff and C. Casanova*. Dept. de Sciences biologiques, Université de Montréal, Montréal, Canada, H3C 3J7.

The objectives of the present study were to examine how the superior colliculus (CS) influences the responses of lateral geniculate cells (LGN). In anesthetized and paralyzed rabbits two tungsten micro-electrodes were aimed at the CS and LGN in order to record single unit activity simultaneously from both structures. An appropriate stimulus (usually a moving light bar) was positioned in the receptive field (RF) of a collicular unit in order to activate the retino-collicular pathway. This stimulus did not modify the spontaneous activity of the geniculate cell. A second stimulus (usually a LED) was flashed in the RF of the latter. Similarly, this second stimulus did not influence the collicular cell under investigation. Thus, the retino-collicular and retino-geniculate pathways were excited separately. The geniculate stimulus was applied at determined intervals after the collicular cell had fired, in this fashion it was assumed that the colliculo-geniculate fibers were activated and produced a modification of the geniculate responsiveness. Results indicated that there was a significant enhancement or decline of geniculate responses when compared to similar but non-contingent stimulation (That is without initial collicular excitation). The influence was strongest when intervals were between 100 and 300 msec corresponding to the latency of a saccade. Control experiments carried out on retinal ganglion cells indicated that the effects were absent at the retinal level. These results support the notion that the CS contributes to the geniculate message relayed to the visual cortex.

Supp. CRSNG and FCAC.

56.19 THE EFFECT OF SUDDEN TACTILE AND AUDITORY STIMULI ON THE VISUAL RESPONSES OF NEURONS IN THE DORSAL LATERAL GENICULATE NUCLEUS OF FULLY AWAKE RABBITS. H. A. Swadlow, T. G. Weyand and P. L. Vera*. Dept. Psychology (U-20), Univ. Conn., Storrs, CT. 06268.

The visual system of the rabbit is especially suited for studies requiring an awake subject because the eye can remain relatively stable for prolonged periods. We have examined the effects of sudden tactile and auditory stimuli upon the visual responses of neurons in the dorsal lateral geniculate nucleus (LGNd) of fully awake, unparalyzed rabbits. All surgical procedures were performed under barbiturate anesthesia several days prior to the first recording session. Principal cells were identified by antidromic activation following electrical stimulation of the visual cortex. Eye position was monitored to an accuracy of 1/3° in early experiments and to within 1/5° in later experiments. We have found that in the fully awake rabbit, sudden auditory or tactile stimulation has a profound effect on the visual response properties of some LGNd neurons. For most concentric sustained cells the response to standing contrast began to diminish appreciably and sometimes disappeared altogether after 2-20 seconds. In most of these cells, however, a sudden auditory or tactile stimulus re-established the response to the visual stimulus at or near its initial high rate. For these cells the auditory and tactile stimulus alone had no effect on the maintained discharge rate of the cell. In order to be sure that this modulation (a) occurred at the LGNd and (b) was not due to subtle eye movements elicited by the arousing stimuli, we tested more than 20 optic tract axons with a sustained, concentric field organization. In no case did we see a facilitation of the visual response following presentation of auditory or tactile stimulus. We conclude that in the fully awake rabbit, the level of arousal has a profound effect on the visual response properties of neurons in the LGNd.

56.20 THE PROJECTION OF THE VISUAL FIELD ONTO THE LATERAL GENICULATE NUCLEUS OF THE FERRET. Kathleen R. Zahs* and Michael P. Stryker. Physiology, Univ. of California, San Francisco 94143

Linden, Guillery, and Cucchiaro (JCN 203:189-211, 1981) have recently presented anatomical findings on the development of the retinogeniculate projection in the ferret, attractive for developmental studies because of the immature state of its binocular visual system at birth. We have begun physiological studies of this charming and gentle animal by mapping the projection of the visual field onto the lateral geniculate nucleus (LGN).

Tungsten microelectrodes were used to record multi-unit and occasional single-unit activity in paralyzed, anesthetized ferrets held in a modified Horsely-Clarke apparatus. Recording sites in vertical electrode penetrations were marked with electrolytic lesions, allowing them to be located in Nissl-stained coronal sections. Although the laminae could be discerned in these sections, they were clearer in autoradiographs prepared after injecting one eye with tritiated proline. At the caudal pole of the LGN, laminae A and A1 took the form of sheets, A1 caudal to A. Rostrally the nucleus was shaped like a "C" and was composed mainly of lamina A.

Receptive fields were plotted at a total of 306 recording sites within the A-laminae in 4 ferrets. All single-unit receptive fields were monocularly driven with ON or OFF centers and antagonistic surrounds. The multi-unit responses were nearly always monocular and were usually of a single center type.

Each LGN represented a single hemifield. The horizontal meridian was found at the dorsal border of the caudal pole of the nucleus and moved ventrolaterally in more anterior sections. The vertical meridian was represented at the medial edge of the dorsal limb of the LGN for the upper visual field and at the medial edge of the ventral limb of the LGN for the lower visual field. Over most of the LGN, lines of projection ran rostrocaudally.

Isoelevation lines took the form of sheets approximately in the plane defined by the rostrocaudal and mediolateral axes. The upper visual field was represented in more dorsal and rostral parts of the LGN; while the lower visual field was represented ventrally and caudally. Isoazimuth lines took the form of sheets in the rostrocaudal and dorsoventral plane, with the more lateral sheets representing the more peripheral visual field. Receptive fields for the two eyes were in register: lines of projection ran caudally from lamina A into A1. The few receptive fields mapped in the C-laminae suggested that lines of projection continued straight through these laminae after passage through A and (sometimes) A1.

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56.21 SYNAPTIC ORGANIZATION OF RETINAL AFFERENTS TO CLASS 1 AND CLASS 2 THALAMO-CORTICAL RELAY CELLS IN THE DORSAL LATERAL GENICULATE NUCLEUS. Toni P. Miles* & Salvatore C. Rapisardi Department of Anatomy, College of Medicine, Howard University, Washington,D.C. 20001

We have investigated the synaptic arrangements between optic tract terminals and two thalamo-cortical relay cells in the later-al geniculate nucleus(DLGN) of the adult cat. It has been demonstrated that the physiology of different relay cell classes is highly correlated with their light microscopic appearance (Friedlander etal '81). Using that morphologic criteria, we identified a Y cell by its Class 1 morphology and a cell that could have either X or Y physiology based on its Class 2 morphology.

Two methods were used in this study to identify cells. The Y cell was reconstructed from electron micrographs of 217 consecutive sections, and identified by its large soma (840um^2) and smooth, appendage-free dendrites. The Class 2 cell was visualized using the gold toning method of Fairen and Peters (J Neurocyto,6 '77) which allowed us to identify the whole cell with the light microscope by its soma size (360um^2), and characteristic appendages at dendritic branching points. A long series (300 consecutive sections) was cut with the added advantage that dendritic profiles in the electron micrographs were marked with gold.

After identification of the two cells, individual terminal boutons of retinal terminals that were presynaptic to them were completely reconstructed. The following differences were found in the way retinal terminals were received by the two cells:(1) the Y cell received retinal terminals on dendritic shafts, while retinal input to the Class 2 cell was restricted to the dendrite appendages, (2) all retinal terminals to the Y cell were found within 30um of the soma. Retinal terminals to the Class 2 cell made contact with appendages that were between 30 and 90um from the soma. There were no retinal contacts made within 30um of the soma on any of the Class 2 cell dendrites studied,(3) Retinal terminals presynaptic to the Class 2 cell made more synapses with flat vesicle profiles than did retinal terminals presynaptic to the Y cell and (4) Retinal terminals presynaptic to the Class 2 cell had almost all of their synapses arranged in a triadic arrangement while only a small proportion of the retinal terminals presynaptic to the Y cell had synapses in this arrangement. A triad is defined by the retinal terminal being presynaptic to both a dendrite and a flat vesicle profile, plus the flat vesicle profile is presynaptic to the same dendrite. Clusters of dendritic appendages were found on the great majority of X cells by Friedlander etal. Our data suggests that triadic arrangements are characteristic of the X system and only to a subpopulation of neurons in the Y system.

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56.22 MORPHOLOGY AND AXONAL TERMINATION OF THALAMIC INTRALAMINAR NUCLEUS (ILN) NEURONS WHICH PROJECT TO VISUAL CORTEX IN SQUIRREL MONKEY. L. Towns, J. Tigges, M. Tigges and L. Walker*. Kirksville College of Osteopathic Medicine, Kirksville, MO 63501 and Yerkes Regional Primate Center and Department of Anatomy, Emory University, Atlanta, GA 30322.

The thalamic intralaminar nuclei (central medial nucleus, CeM, and paracentral nucleus, PC) have been shown to project to the visual cortex in squirrel monkey (Tigges, et al., in press). In order to further assess the morphology and connections of these thalamocortical neurons, three squirrel monkeys received massive HRP injections in either the occipital lobe or in area MT. After a 72 hour survival period, each animal was perfused under deep anesthesia and the thalami were sectioned and processed by either TMB or DAB protocols to reveal the retrogradely transported HRP.

In coronal sections, numerous labeled somata are seen to extend in a continuous arc from CeM ventromedially into the PC dorsolaterally. Furthermore, two separate, concentric groups of filled cells are seen in PC, one medially and one laterally placed. In both CeM and PC, the labeled somata are round or spindle shaped and typically have two or three principal dendritic processes; the somata and proximal dendrites are oriented parallel to the fibers of the internal medullary lamina of the thalamus in which they are embedded. Generally, the filled neurons of the CeM are smaller and rounder than the labeled neurons in PC which become larger and more elongated as this nucleus passes dorsolaterally. A few very large,well-filled cells are seen in the lateral division of PC. These prominent neurons exhibit a much more diffuse and richly branching dendritic tree than do other neurons in the intralaminar complex.

In a preliminary study, [^{3}H]-amino acids were injected bilaterally into the ILN and the termination of these projections in the visual cortex was studied autoradiographically. The principal site of termination of the ILN afferents was in layers V and VI over a large expanse of the occipital visual area. Some light termination was occasionally seen in layers I-III but lamina IV received no input from ILN.

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56.23 LIGHT AND E.M. LOCALIZATION OF CYTOCHROME OXIDASE STAINING IN THE LATERAL GENICULATE NUCLEUS. G. H. Kageyama* and M. Wong-Riley (SPON: C. Brown). U. of Calif. San Fran. CA 94143 and Med. Coll. of Wisconsin, Milwaukee WI 53226.

In the lateral geniculate nucleus (LGN) of the cat, physiologically defined classes of cells can be distinguished on the basis of cell size and distribution. In order to determine whether the different cell types as well as specific neuronal profiles (e.g., dendrite, axon, axon terminal) have distinct levels of metabolic activity, sections of cat LGN were reacted histochemically for cytochrome oxidase (CO) and examined at the light and E.M. levels. In the cat lamina A and A1 were highly reactive, while lamina C was only moderately so. Predominantly large but also a few medium sized intensely reactive neurons were observed within lamina A, A1 and C, and along the interlaminar zones. The size and distribution of the large reactive cells were similar to those reported for type 1 cells and physiologically recorded Y-cells. At the E.M. level, the majority of the intensely reactive neurons were large, confirming our light microscopic observations. In the neuropil, most of the highly reactive mitochondria were found within dendritic profiles, especially those closely associated with synaptic glomeruli. RL axon terminals were almost always nonreactive, while presynaptic profiles with flattened or pleomorphic vesicles (F1, F2 and FD) usually contained one or more moderate to highly reactive mitochondria. The RS terminals often did not contain any mitochondria. When they did, they were usually light to moderately reactive.

In the LGN of cats monocularly deprived at birth, notably fewer reactive neurons were observed in the deprived A, A1 and C laminae and they were small to medium in size. The absence of large reactive neurons within the deprived lamina may be related to the reported paucity of recordable Y-cells from these layers.

Our light microscopic findings imply that large (presumed Y) cells have higher levels of CO activity and that these cells are dramatically affected by early monocular deprivation. Our E.M. findings suggest that specific neuronal profiles in the cat LGN have distinct levels of CO staining. The localization of many intensely reactive mitochondria mainly within dendrites (but also F terminals) indicates that these structures are metabolically very active.

Preliminary data on the primate (macaque and squirrel monkey) LGN suggest that the larger (magnocellular) neurons are more reactive for CO than the medium and small (parvocellular) neurons. However, the dorsal parvocellular lamina 6, which contains both small and medium sized reactive cells, also appears to have elevated levels of CO staining. As in the cat LGN, most of the CO activity in the neuropil was localized within dendrites and F terminals, while RL terminals were consistently nonreactive.

56.24 Behavioral Analysis of Embryonic Neocortical Transplants into the Lateral Geniculate Nuclei in the Rat, R.B. Wallace, G.D. Das*, J. Harnsberger*, J. Gustafson*, Douglas Ross*, Richard Smeyne* and James Thompson*, Lab. of Developmental Psychobiology, University of Hartford, West Hartford, CT 06117.

Prior research has indicated that embryonic neocortical tissue may be successfully transplanted into the cerebella of juvenile host animals (Wallace, R.B. and Das, G.D., Brain Research in press). These studies indicated that although the growing transplants replaced up to 80% of the host cerebellum, these animals were indistinguishable behaviorally from control subjects. Anatomical evaluation indicated that the transplants were fully integrated with the host neuropil. In an effort to extend these results to a sensory system, the following experiment was conducted. Embryonic neocortical tissue from 17 day embryos was transplanted bilaterally into the lateral geniculate nuclei of 10 day old host animals (Long-Evans hooded rats). These animals were then behaviorally compared with animals that had received bilateral LGN lesions and with normal control subjects. Tests employed consisted of Open Field, Neurological Exam, Visual Cliff and an operant brightness task. Following behavioral testing all animals were perfused transcardially with buffered formalin, the brains embedded in the coronal plane, sectioned and stained with H&E for microscopic examination. Microscopic evaluation indicated that in the transplant animals, the neocortical tissue had displaced and replaced the LGN bilaterally and, in some cases, much of the neighboring diencephalic tissue as well. Analysis revealed, however, that in the lesioned animals, the projections from the LGN were spared due to misplacement of the lesions. These data find support in the behavioral analyses which suggested no significant differences between the groups on any of the tasks employed. The point of interest, however, is the fact that there were no behavioral differences between the transplant and control animals although the growth of the transplants had replaced substantial amounts of tissue including the LGN. Additional work remains to be done.

57.1 COUNTS OF SYNAPSES ON IDENTIFIED NEURONS IN THE MOUSE CORTEX. A. Schüz*, A. Münster*, M. Dortenmann* (Spon: C. Wehrhahn). Max-Planck-Institut f. Biol. Kybernetik, Tübingen, W. Germany

Anatomical measurements and theoretical considerations have led to an estimate of a synaptic density of 1 synapse every 1 to 4 µm of axonal length (Braitenberg, 1981, Adv. Physiol. Sci. Vol. 16, Grastyán, Molnár, eds.). This number has been deduced from a synaptic density of $10^9/mm^3$ and an axonal density of 1-4 km/mm^3. We have now traced electronmicroscopically parts of the axonal tree of a Golgi-impregnated pyramidal cell. Its cell body was situated 325 µm below surface on the dorsolateral convexity of the hemisphere. The method consisted in a combination of Golgi-Colonnier, the EM-procedure of Blackstad with slight variations, and the addition of PTA. In spite of an insufficient preservation of the surrounding tissue, collaterals can be followed without loss of synapses. Tracing 70 µm of a collateral beginning at its origin, 17 synapses were found. This number (1 synapse every 4 µm) corresponds to the predicted value. Interestingly, the synapses were not distributed evenly. They were concentrated on 44 µm leaving long pieces at the origin of the collateral free of synapses.

A second point concerns the number of synapses on stellate cell dendrites, measured on PTA-stained material. An intentionally rough treatment of the tissue (acid fixative, exposition to ultrasound) led to a rather selective darkening of stellate cell dendrites. It induced, furthermore, the destruction of mitochondria which made the stain still more selective for synapses and allowed relatively thick sections (2000 Å) to be used. The pictures give a realistic impression of the synaptic density on stellate cell dendrites. By a reasoning akin to the Abercrombie-correction we estimated a density of 3 synapses per μ^2 of dendritic surface or 5 synapses per µ of dendritic length (on dendrites with a diameter of 0.5µ). The density is thus higher than the density of synapses on pyramidal cells (1.5 spines/µ). This shows clearly that the role of spines must not be seen in an increase in the number of possible synapses.

The synapses along a stellate cell dendrite are crowded more densely than in the rest of the tissue. This makes one think that at a certain stage stellate cell dendrites act as attractors for presynaptic elements.

57.2 A STUDY OF RECIPROCAL CONNECTIONS OF CORTICORETICULAR CELLS WITH BRAINSTEM NUCLEI USING ANTEROGRADE AND RETROGRADE TRANSPORT OF WGA-HRP IN CATS, J.I. Franck and R.P. Dum.
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We have determined the exact sources of the reticular formation (RF) projections from frontoparietal cortex, and studied the afferent and efferent connections of these same cortical regions with the brainstem and diencephalon.

Ten cats were injected with 2-3 doses of 0.05-0.10µl of 2% WGA-HRP. The tissue was processed using the TMB method after a 2-3 day survival time. Injections in the pontine or medullary RF resulted in heavy retrograde labelling of cortical neurons in the presylvian (PS) gyrus and area 6 (esp. 6aβ) and moderately heavy labelling of rostral anterior ectosylvian gyrus (AEG-SII face area). Only scattered labelling of area 4,3,1 and 2 was noted.

Injections of WGA-HRP into area 6aβ,PS gyrus, or rostral AEG-SII were made in order to map the sub-cortical inputs and outputs of those areas. Densely retrogradely-labelled extra-thalamic nuclei (n.) which project to all of those cortical regions included the ventral tegmental area of Tsai, n. of the fields of Forel (FF),PAG, substantia innominata, and the raphe, coerulear-parabrachial (LC-BCM), and hypothalamic complexes. Smaller projections originated from the subthalamus, n. of the stria terminalis, and RF. Many thalamic n. were heavily labelled, in common, by all cortical injections. These included the intralaminar complex, VA, VL and VMP. In particular, the MD and paraventricular thalamic n. projected to area 6aβ and PS gyrus; and VMB and VBA to the AEG-SII face area.

After all cortical injections, heavy anterogradely labelled terminal fields were found in the pontine gray, caudate, RF, n. of Darkschewitsch, interstitial n. of Cajal, superior colliculus and pretectal n. The AEG-SII face area injection resulted in heavy trigeminal n. labelling. Projections to the tegmental reticular and lateral reticular n. from area 6aβ and PS gyrus were also observed.Dense reciprocal terminal fields were found in all cortically projecting thalamic nuclei and some extrathalamic nuclei including FF and PAG.

This study demonstrated the efficacy of WGA-HRP in the mapping of both afferent and efferent projections. We conclude that: 1) The RF is not a major relay from area 4 to the spinal cord, but rather receives projections from accessory motor and somatosensory regions; 2) Thalamic n. that project to these cortical regions receive heavy reciprocal cortical projections; 3) Many extrathalamic inputs to these cortical regions exist, some of which receive dense, and others, light reciprocal cortical projections; and 4) Many of the extrathalamic n. that receive these cortical projections are known to be involved in the control of eye and head movements.

57.3 AFFERENT AND EFFERENT CONNECTIONS OF SOMATIC MOTOR CORTEX RELATED TO TONGUE CONTROL IN THE RAT. L.D. Aldes*. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77025. (SPON: J.F. DeFrance)

As part of a larger investigation into the neural substrates subserving tongue control in the rat, the organization of afferent and efferent connections of somatic motor cortex related to the tongue was studied with retrograde (HRP) and anterograde (autoradiography) tracer techniques. Microiontophoretic injections of either HRP or tritiated amino acids (leucine, lysine and proline) were made into discrete cortical foci which, upon intracortical microstimulation, yielded the lowest threshold flick responses of the tongue.

Following injections of HRP, retrogradely labeled neurons were found in widely divergent sites including motor and sensory cortex, deep telencephalic nuclei, thalamus, ventral forebrain, hypothalamus, subthalamus and brainstem. The heaviest labeling of cells was seen in the ventrolateral (VL), ventromedial (VM) and posterior (PO) nuclei of the thalamus. Labeled cells were organized into longitudinal columns and occupied distinct topographic regions in each nucleus. Other heavily labeled areas included the contralateral motor and ipsilateral sensory and "taste" cortex; the centrolateral (CL), paracentral (PC) and central medial (CeM) intralaminar nuclei of the thalamus and the dorsal raphe. Moderate labeling of cells in the ventral forebrain and hypothalamus included the substantia innominata, substriatal gray, lateral and magnocellular preoptic nuclei, while distinct cell labeling also was evident in the entopeduncular and subthalamic nuclei, zona incerta, basolateral amygdala, parafascicular nucleus and locus coeruleus. Sparse labeling of cells was found in the midbrain tegmentum, medial parabrachial nucleus, nucleus centralis superior, caudoputamen (CP) and other nonspecific thalamic nuclei.

The course and distribution of labeled axons following injections of tritiated amino acids revealed a correspondingly diverse pattern of efferent connectivity. While reciprocal corticocortical, corticothalamic and corticosubthalamic projections were found, a dense, topographically organized, bilateral, corticostriate projection to the lateral one-third of the CP was seen. Moderately dense projections to the ipsilateral substantia nigra and lateral midbrain tegmentum were observed, while labeled axons were seen bilaterally in the bed nucleus of the anterior commissure and brainstem reticular fields.

Results of this study have demonstrated the diverse nature and topographic organization of both input and output of the somatic motor cortex related to the tongue. These data are necessary for understanding the neural basis of tongue control.

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57.4 AN AUTORADIOGRAPHIC STUDY OF THE AMYGDALOCORTICAL PROJECTIONS IN THE MONKEY. D.G. Amaral and J.L. Price (SPON: B. Claiborn). The Salk Institute, LaJolla, CA; Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO.

As part of ongoing studies of the connections of the primate amygdaloid complex, we have examined amygdalocortical projections autoradiographically in a series of 9 cynomolgus monkeys prepared with single 100µl injections of ^{3}H-amino acids. In 7 of these monkeys bilateral injections were made (aimed at different targets) so that 16 injections were available for analysis; all major divisions of the amygdaloid complex were involved by at least one injection. These experiments demonstrated very substantial projections to the same cortical areas which innervate the amygdala. Moreover, the distribution of these projections varied depending on the amygdaloid nucleus labeled.

The lateral nucleus projects primarily to temporal and insular cortex. All divisions of temporal neocortex (TA, TE, TG and TF-TH) receive input from the lateral nucleus. The ventral portion of the insular cortex is also labeled for much of its rostro-caudal extent. The basal (or lateral-basal) nucleus sends a restricted projection to the rostral, agranular insular cortex and a very few fibers to the temporal neocortex (only to TG) but heavily innervates orbitofrontal and cingulate cortex. The magnocellular division projects most heavily to the medial and lateral orbitofrontal cortex (especially Walker's areas 14 and 12) whereas the parvocellular division projects most heavily to the rostral cingulate cortex (areas 24 and 25). There is a much lighter projection to the regions dorsal to the cingulate cortex including areas 6, 8 and 9. The magnocellular division of the basal accessory nucleus projects to the orbitofrontal cortex and insular cortex while the parvocellular division appears to send fibers to all portions of both the insular and temporal cortices. In all these areas the terminal labeling is concentrated in the deep part of layer I and in layer II, and, in many cases, also in layers V and VI.

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57.5 THALAMIC AFFERENTS TO THE CORNEAL AREA OF THE PRIMARY SOMATOSENSORY CORTEX OF THE RABBIT. D.C. Nathanson* and C.F. Cegavske. Department of Psychology, SUNY-Binghamton, Binghamton, NY 13901.

The neural connections from the thalamus to the primary somatosensory cortex (SI) that mediate sensory information from the cornea have not been previously localized. These neurons were identified by first using averaged evoked potentials (AEPs) from the surface of the skull to localize SI, then injecting horseradish peroxidase (HRP) into the underlying corneal area of the cortex.

Evoked potentials were recorded differentially, from 7 adult New Zealand white rabbits anesthetized with Chloropent, and averaged using a Nicolett signal averager. Stainless steel tubes (27 gauge) filled with electrode paste and spaced 3 mm apart were used as electrodes. Stimulation consisted of three 0.5 msec pulses at 1 kHz administered through wound clips in the skin (contralateral forelimb, hindlimb, ear, and vibrissa region) and gold-ball electrodes on the corneal surface. SI was localized using AEPs from all stimulus points. The maximal AEP to corneal stimulation in all animals was consistently found to lie in an area under the squamosal bone 2.5-4.5 mm posterior to bregma and 1.5-4.0 mm ventral to the intersection of the squamoparietal and coronal sutures. The point of maximal AEP was located in individual animals and a hole was then drilled in the skull to allow injection of HRP 0.5 mm below the cortical surface.

Pressure injections of 0.03-0.10 µl 30% HRP (Sigma, Type VI) were made through glass pipettes (tip diameters 25-40 µm). Four brains were injected at the site of maximal response to corneal stimulation, 2 brains injected 2 mm anterior to this site and one 2 mm posterior to it. Brain sections were processed using TMB.

Injections into the corneal area of the cortex produced labeling in the contralateral cortex, the arcuate division of the ventrobasal complex (VBC), nucleus reticularis; and in some animals, the centromedian nucleus, the posterior nucleus, and the magnocellular region of the medial geniculate.

The labeling in the arcuate was in a compact group of cells beginning at the caudal pole of the VBC and extending rostrally for about 2.0 mm forming 2 or 3 horizontally-oriented medially-curving fingers. In coronal sections the fingers were crescent shaped (concave medially). It is concluded that the neurons which mediate sensory information from the cornea are contained within this labeled group of cells. This conclusion is supported by the results from the injections anterior and posterior to the point of maximal corneal AEP. They labeled similar-shaped groups of cells adjacent but ventromedial and dorsolateral respectively to the corneal representation. These areas overlapped the corneal area by about 10% at their mutual borders.

57.6 LONG-TERM HABITUATION PRODUCES A DECREASE IN CEREBRAL METABOLISM IN RAT FIRST SOMATOSENSORY CORTEX. C. L. Hand* and P. J. Hand (SPON: J. Metzler). Dept. of Animal Biology, Sch. of Vet. Med. and Inst. of Neurological Sciences, University of Pennsylvania, Phila., PA 19104

Using the quantitative (^{14}C)-2-deoxyglucose (2-DG) method of Sokoloff et al (J. Neurochem. 28, 1977), we designed a sensory "enrichment" paradigm to evaluate chronic effects of increased receptor activation on metabolic (functional) organization in first somatosensory cortex (SI) of developing and adult CNS. The rat vibrissa-cortical "barrel" system was used as a model because of its precise and unilateral central projections (terminating in contralateral SI), permitting use of ipsilateral SI as an internal control (matched pair). Both rat pups (n=7) and adults (n=3) were used, with stimulation repeated for 90 and 60 days, respectively. Rats were hand-held and gently restrained by wrapping. A central vibrissa (#3, row C: C3) in an intact vibrissa field was stroked in a rostrocaudal plane at 4-5 Hz using a mechanical stimulator. Length of stimulation sessions was constant at either 5, 10, or 15 min/day (3 groups). In all cases, the intersession interval was at least 24 hours, and stimulus intensity, stimulus frequency, and plane of stroking were held constant. The experimental side was randomly chosen to control for any natural brain asymmetries. After 60 or 90 days' stimulation, experimental and control C3 were stroked at 4-5 Hz following an I.V. pulse injection of 30 uCi (^{14}C)-2DG, and the tissue prepared for quantitative analysis. Data generated from serial tangential autoradiographic images of the C3 functional column in SI indicate a small, extremely consistent decrease in metabolic labeling in the repeatedly activated C3 column as compared to the control C3 column: mean decreases of 3-9.5% in 9 out of 10 matched pairs. Laminar comparisons were more revealing: (1) Supragranular laminae (I-III): 10.3% mean decrease below control labeling. (2) Lamina IV: 7.5% mean decrease below control. Using the Wilcoxon matched-pairs signed-rank test, results are highly significant for both supragranular laminae and lamina IV ($p \leq .005$). (3) There was no significant difference in labeling in laminae V-VI. In general, labeling in the repeatedly activated C3 column tended to be less focal (less discrete) than the corresponding control C3 columnar labeling.

These preliminary findings suggest development of long-term habituation in SI cortex with repeated weak stimulation. Current efforts are directed at analyzing: (1) the contribution, if any, by other levels of this pathway, and (2) age and time course effects on the observed phenomenon. (Supported by USPHS grant NS 14935)

57.7 DIVERGENT CORTICAL PROJECTIONS OF THE MEDIAL PULVINAR NUCLEUS: A RETROGRADE FLUORESCENT TRACER STUDY IN THE MONKEY. C. Asanuma, R.A. Andersen and W.M. Cowan. The Salk Institute, La Jolla, CA 92037.

Following large, single or multiple injections of the dye fast blue (FB) into the inferior parietal lobule (IPL) of cynomolgus monkeys, retrogradely labeled cells are observed in the following thalamic nuclei: Pul.m., SG, Li, Pul.o., Pul.l., CL, MD, LD, LP and VLps (Olszewski's 1952 abbreviations). The labeling in the medial pulvinar nucleus (Pul.m.) is both dense and highly organized. In frontal sections, two or three disk-like aggregations (approx. 300 µm in the shorter dimension) of labeled neurons, separated by label-free areas, are consistently seen. The disks are oriented from dorsomedial to ventrolateral within Pul.m. and extend throughout much of the anteroposterior dimension of the nucleus. After IPL injections of ^{3}H-amino acids similar disk-like patterns of anterogradely transported label are seen in autoradiographs, which suggests that there is a high degree of reciprocity in the cortico-thalamic and thalamo-cortical projections between the Pul.m. and the IPL. Following large multiple injections of the dye nuclear yellow (NY) into areas 8, 45 and 46 of the prefrontal cortex, retrogradely labeled neurons are observed in: Pul.m., MD, SG, Li, CL, Pcn, LD, LP and VLps. The density of NY labeling is highest in MD and Pul.m. The NY labeled cells in MD are fairly uniformly distributed, but those in the Pul.m. are arranged as a single disk-like aggregate with the same dorsomedial to ventrolateral orientation as the IPL projecting disks. No cytoarchitectonic correlates of these cell groupings in Pul.m. are evident in Nissl-stained sections.

To determine the exact relation of the two Pul.m. projections, multiple injections of NY were made into the lateral prefrontal cortex (LPC) 11 days after multiple injections of FB into the IPL in the same hemisphere. In these brains the disk-like aggregations of FB and NY labeled cells are found to overlap extensively, although the FB cells tend to be concentrated laterally and the NY cells medially. A complete intermingling of FB cells and NY cells is evident in the overlap zone; however, only a small proportion (< 0.1%) of the cells is doubly-labeled. Following these dual tracer injections, label-free areas between the disks are still apparent.

These observations indicate that there are groupings of neurons within the Pul.m. which project to both the IPL and the LPC, but that within these groupings the cells that project to the IPL and to the LPC comprise largely independent populations of neurons. The relation of these thalamic projections to those that project to the temporal lobe (Siqueira '65) remains to be investigated.

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57.8 OBSERVATIONS ON THE CALLOSAL AND ASSOCIATIONAL CORTICO-CORTICAL CONNECTIONS OF AREA 7A OF THE MACAQUE MONKEY. R.A. Andersen, C. Asanuma and W.M. Cowan. The Salk Institute, La Jolla, CA 92037.

As part of a detailed study of the connections of the inferior parietal lobule we have used anterograde autoradiographic and retrograde fluorescent-dye tracing techniques to examine the callosal and associational connections of cortical Area 7a (PG) in the cynomolgus monkey Macaca fascicularis.

To determine whether the same or different neurons give rise to callosal and associational projections, large, multiple injections of the dyes fast blue and nuclear yellow were made into area 7a of the contralateral side and into the ipsilateral prefrontal cortex (Areas 8,46 and 45 of Walker). These experiments indicate that for these two cortical fields the two projections arise largely from separate populations of neurons; although the two populations overlap and have similar laminar distributions. Less than 0.05% of the cells of these two intermingled populations were doubly-labeled in these experiments.

Area 7a receives a substantial visual input from several locations in the prestriate cortex (Area 19 or OA); the major projection is from patches of cells in the prelunate gyrus.

The double retrograde dye experiments indicate that the populations of neurons which project to ipsilateral Area 7a and to the ipsilateral prefrontal cortex from the cingulate cortex and the from the superior temporal sulcus are partially overlapping and intermingled. But again, these projections arise largely from separate populations as indicated by the paucity of double labeled neurons.

Single restricted injections of tritiated amino acids in Area 7a produced discontinuous and widely spaced "columns" of label in the contralateral homotypic cortical field; however, the strongest labeled columns were always at positions mirror symmetric to the injection site. The discontinuity of terminal labeling was less distinct after multiple amino acid injections. Single or multiple injections of the fluorescent dyes produced a more or less continuous pattern of labeled cells in the contralateral area 7a. Single injections of either anterograde or retrograde tracers produced a patchy pattern of intracortical label within the ipsilateral area 7a. This intracortical projection involves all of the cytoarchitectonic field PG including the anterior bank of the superior temporal sulcus.

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57.9 THALAMOCORTICAL PROJECTIONS TO THE LATERAL PREFRONTAL CORTEX AND ADJACENT CORTICAL AREAS IN RATS AND CATS. S.J. Wiegand and J.L. Price. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Recent studies have suggested that significant species differences may exist in the organization of the projection from the mediodorsal nucleus of the thalamus (MD) to the lateral prefrontal cortex (PFC) (e.g., Divac et al., JCN 180:59, 1978; Markowitz and Pritzel, Physiol. Psychol. 7:1, 1979). This possibility has been difficult to evaluate as conflicting reports have been published regarding the organization of MD efferents to the cortex within species. The present experiments examine this question in the rat and cat.

Small injections of wheat germ agglutinin labeled with horseradish peroxidase (0.5-1.0% w/v) were placed over the entire extent of the lateral PFC and within adjacent cortical areas in rats (8-35 nl) and cats (50-100 nl). Analysis of retrogradely labeled cells in thalamus demonstrates that the organization of MD efferents to the lateral PFC is nearly identical in rat and cat. In both the lateral part of the MD projection field extends from the frontal pole of the hemisphere caudally to the level of the posterior agranular insular cortex. It is bounded ventromedially by the ventrolateral orbital cortex which receives an input from the submedial nucleus of thalamus and more caudally by the piriform cortex. Dorsolaterally it is bounded successively by premotor cortex (area 6), gustatory cortex (G) and by dysgranular and granular insular cortices (I_d and I_g). Area 6 and G receive their principal thalamic inputs from the ventrolateral nucleus and basal ventromedial nucleus respectively.

At least 5 subdivisions of the MD projection field in the lateral PFC can be identified using connectional and cytoarchitectonic criteria. Most rostrally the lateral frontal polar cortex (FP_1) occupies the entire dorsoventral extent of the PFC and receives a projection from the lateral, paralamellar portion of MD. More caudoventrally the lateral orbital cortex (LO) and ventral agranular insular cortex (AI_v) receive projections from the central and dorsomedial portions of MD, respectively. Dorsal to these areas the dorsal lateral orbital cortex (DLO) and dorsal agranular insular cortex (AId) receive input from the ventral and the caudal ventral medial parts of MD, respectively.

All subdivisions of the lateral PFC as well as the surrounding cortical areas receive projections from the principal part of the ventromedial nucleus (VM) in both rats and cats. Retrograde labeling of the midline and intralaminar nuclei is also evident in both species. In the cat the supragenicular complex projects heavily to I_g and I_d and also to FP_1, DLO and AId. An equivalent projection was not found in the rat.

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57.10 VENTROANTERIOR NUCLEAR CONTRIBUTIONS TO VISUAL CORTEX IN THE RAT. R. Rieck and R.G. Carey. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, Arizona 85013.

Several previous reports from this and other laboratories have shown that intralaminar nuclei terminate in the infragranular layers of neocortex, and that the thalamic ventromedial nucleus projects to cortical layer I (Herkenham, '79, '80; Rieck and Carey, '82; Rieck, Carey and Neal, '82). The present report reevaluates the actual organization of the rostral thalamic constituents that project to layer I in the rat.

Following layer I HRP applications (Carey, '79) in visual cortices and sectioning of the brain in the coronal plane, intense cellular labeling is seen in the ventromedial (VM) nucleus, dorsomedial rim of the ventrolateral nucleus (VL), and the anteromedial nucleus (AM). The labeled cell types throughout the VM and VL nuclei include large multipolar and fusiform neurons, and they are located in an AChE-negative zone of the thalamus. When the same experimental procedure is followed except that the brain is cut in the horizontal plane, extensive neuronal labeling is seen in the rostral cap of the thalamus. Retrogradely labeled neurons are found lateral to the mammillothalamic tract and form a gentle arcade that extends to the lateral limit of the thalamus. These labeled neurons similarly are large multipolar and fusiform cells, and lie in an AChE-negative zone. This zone of retrogradely labeled neurons has been described as the ventroanterior (VA) nucleus of the thalamus (Scheibel and Scheibel, '69), and we suggest that it coincides exactly with the labeled region that has been defined as the VM nucleus, and the dorsomedial part of the VL nucleus of the rostral thalamus. These similarities lead to the suggestion that this intricate system of layer I afferents arises from cellular aggregates in the thalamus that are actually components of the VA nucleus. This region of the thalamus also has been shown to receive nigral, entopeduncular, and deep cerebellar afferent terminations, and, therefore, these extrapyramidal nuclei may be capable of modifying activity in a wide range of cortical areas by means of a diffuse layer I projection system that is relayed by the ventroanterior nucleus.

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57.11 DISTRIBUTION AND THALAMIC ORIGIN OF ACETYLCHOLINESTERASE ACTIVITY IN RETROSPLENIAL CORTEX OF THE RAT. Leslie Ann Tengelsen* and Richard T. Robertson. (Sponsor: Earle A. Davis, Jr.) Department of Anatomy, College of Medicine, University of California, Irvine, CA. 92717.

The results of a variety of experiments are presented to demonstrate that the presence of distinct acetylcholinesterase (AChE) activity in ventral retrosplenial cortex is dependent on the existence of a thalamocortical projection from the thalamic anterior dorsal nucleus (AD). AChE activity in normal and experimentally manipulated hooded rats was determined histochemically by a modified version of the method of Koelle; non-specific cholinesterases were inhibited by 10^{-5}M iso-OMPA.

In normal rat, extra-somal AChE activity occurs in two distinct bands in layers I and III throughout ventral retrosplenial cortex (VRS, areas 29b and 29c). The laminar pattern of AChE activity is similar to the pattern of thalamocortical terminals in VRS from AD (Robertson and Kaitz, JCN, 1981, 195:501). Neurons in AD thalamus also display strong AChE activity. Placement of unilateral lesions that involve most of VRS result in severe retrograde degeneration of neurons and loss of AChE activity in ipsilateral AD. When injections of horseradish peroxidase (HRP) are placed in VRS and the tissue processed for both AChE and HRP histochemistry, double labeled somata are seen commonly in AD. Placement of unilateral electrolytic lesions in AD results in a marked reduction in AChE reaction product in layer III of ipsilateral VRS, but no apparent change in AChE activity in layer I. Lesions placed in medial and intralaminar thalamic nuclei, not involving AD, do not affect AChE staining in VRS. Similarly, lesions placed in the basal forebrain that include the nucleus of the diagonal band and medial septum do not produce a detectable change in histochemically demonstrable AChE activity in layer III of VRS.

These results are interpreted to indicate that the presence of AChE activity in layer III of VRS is dependent on the existence of a thalamocortical pathway that originates in AD thalamus.

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57.12 IBOTENIC ACID LESIONS OF THE PRIMATE NUCLEUS BASALIS OF MEYNERT RESULT IN DECREASED CORTICAL CHOLINE ACETYLTRANSFERASE. R. G. Struble*, M. McKinney*, P. R. Sanberg, C. A. Kitt, M. R. DeLong, J. T. Coyle and D. L. Price*. Departments of Pathology, Neurology, and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

In Alzheimer's disease (AD), levels of presynaptic cholinergic cortical markers (choline acetyltransferase [CAT] and acetylcholinesterase [AChE]) are severely decreased when compared to age-matched controls. The primary source of cholinergic innervation to cortex is believed to be the nucleus basalis of Meynert (nbM), made up of large, chromophilic, AChE-rich neurons and known to project directly and diffusely to cortex. It has been suggested that the severe depletion of this nerve cell population underlies the cortical cholinergic deficit in AD. Lesions of the rodent homologue of this nucleus, located in the ventral globus pallidus, result in a major loss of cortical cholinergic presynaptic markers. Because similar experiments have not been performed in monkeys, which have an nbM more comparable to that in the human, we designed experiments to selectively ablate this nucleus and to correlate the location and extent of the lesion with activities of presynaptic cholinergic markers in cortex. In cynomolgus monkeys, electrophysiological techniques were used to locate the nbM neurons, which show distinctive firing patterns, and these cells were ablated by injecting ibotenic acid, a toxin which destroys neurons while leaving terminals and fibers of passage intact. Animals were allowed to survive seven days after the lesion. Fifteen bilateral samples of cortex were taken and rapidly frozen; subsequently, the brains were perfused, immersed in formalin, and processed for cresyl violet staining and AChE histochemistry to demarcate the lesion and to examine changes in AChE staining in the cortex. Frozen tissues were analyzed for CAT activity with the activity from the hemisphere ipsilateral to the lesion compared to homologous samples from the contralateral side. Our results show that there were significant reductions of cortical CAT activity ipsilateral to the nbM lesions and that lesions, in different parts of the nbM, resulted in different patterns of reduced cortical CAT activity. These studies are consistent with the concept that pathological changes in the nbM represent, in part, the substrate for the well-documented deficits in cortical presynaptic cholinergic markers in AD. (Supported by NIH NS 07179 and MH 15330.)

57.13 THE NUCLEUS BASALIS OF MEYNERT: PROJECTIONS TO THE CORTEX, AMYGDALA, AND HIPPOCAMPUS. C. A. Kitt, D. L. Price*, M. R. DeLong, R. G. Struble*, S. J. Mitchell, and J. C. Hedreen. Neuropathology Laboratory, Departments of Neurology and Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The efferent projections of neurons contained within the nucleus basalis of Meynert (nbM) of the rhesus monkey were examined by autoradiographic tracing techniques. In primates, nbM neurons are enriched with acetylcholinesterase (AChE) and may provide a large proportion of cholinergic innervation to cortex. The present series of experiments were designed to determine the projections of nbM neurons to cortical and subcortical structures.

Guided by electrophysiological recording, stereotaxic injections (1-2.5µl) of [^{3}H] amino acids were placed in the nbM. Our results show that the nbM projects to all regions of cortex. Dense accumulations of silver grains were present within the cingulate, insular, temporal, frontal, entorhinal, parahippocampal, and subicular cortices, as well as the claustrum; the parietal and occipital cortices were lightly labeled. In addition, the nbM projected to the amygdaloid complex and the hippocampal formation. The basolateral nucleus of the amygdala contained the greatest accumulation of silver grains, although other amygdaloid nuclei were labeled as well. Within the hippocampus, label was observed over all segments with some variation in density. In rostral sections, labeled efferents from the nbM passed medially between the medial septal nucleus and the diagonal band of Broca. At the level of the nbM, efferents exited laterally and passed within the external and extreme capsules to enter the white matter of all cortical lobes. The intensity and laminar distribution varied in different cortical regions. The pattern of silver grains in the cortex, amygdala, hippocampus, and above-described fiber pathways correlate well with the pattern of AChE staining in these regions. These studies demonstrate, for the first time by the anterograde tracing technique, the pathways by which AChE-positive axons derived from the nbM innervate the cerebral cortex and other telencephalic nuclei in Rhesus monkey.
(Supported by NIH NS 07179).

57.14 ELECTROPHYSIOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF NEURONS THE NUCLEUS BASALIS OF MEYNERT IN MACAQUE MONKEYS.
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The nucleus basalis of Meynert (nbM) in the primate consists of large neurons located primarily in the subpallidal region of the basal forebrain but also within the medullary laminae of the globus pallidus and along the anterior commissure and internal capsule. The neurons of the nbM, which send cholinergic projections to the cerebral cortex, are selectively depleted in the brains of Alzheimer's patients. In a previous study, neurons in the more caudal portions of the nbM and within the laminae exhibited discharge patterns different from those of pallidal neurons and were found to be related primarily to delivery of a juice reward. The present study was undertaken to further characterize the neurons of the nbM, particularly those located rostrally.

The activity of single neurons was recorded in macaque monkeys during periods when the animal 1) sat motionless, 2) actively reached for juice, food, and non-food objects, and 3) received drops of juice delivered through a spout. In addition, neuronal responses to passive manipulation of individual body parts were studied. At the end of the experiments, the locations of the cells were confirmed histologically. The majority (80%) of nbM neurons (n=59) had one of two types of discharge patterns in the resting animal. The most common type (36 of 59 cells) had a regular spontaneous discharge (mean rate 19/sec, range 6-47/sec) with modal interspike intervals from 20-65 msec. A second type (11 of 59 cells) had a slower discharge rate (mean 7/sec, range 3-11/sec) with modal interspike intervals from 80-350 msec. The mean spike duration of nbM neurons was 214 usec. By contrast, the mean spike duration of pallidal neurons was 154 usec.

As was found in the previous study, a large proportion (83%) of nbM neurons tested responded phasically to delivery of juice. In addition, 57% responded phasically to reaching for juice. Approximately half of the neurons that showed responses during reaching tasks responded during reaching for non-food objects. Many cells (39%) exhibited non-specific changes in discharge during examination and testing. Very few (6%) cells did not respond during any of the paradigms or manipulations. None of the cells studied exhibited responses to passive manipulations of individual body parts.

These studies indicate that nbM neurons in the behaving primate exhibit responses to a wider range of behaviors than reported previously. Furthermore, nbM neurons show clear phasic changes in discharge rates during behavioral tasks.
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57.15 A FIBER NETWORK IN MONKEY CEREBRAL CORTEX REVEALED BY ACETYLCHOLINESTERASE IMMUNOCYTOCHEMISTRY. J. C. Hedreen, G. R. Uhl, S. J. Bacon*, C. L. White III*, D. L. Price*, and D. M. Fambrough. Neuropathology Laboratory, Departments of Pathology and Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; Department of Embryology, Carnegie Institution of Washington, Baltimore, MD 21210

Monoclonal antibodies to human erythrocyte acetylcholinesterase (AChE), known to react at human and monkey neuromuscular junction (Fambrough et al., Proc. Natl. Acad. Sci. USA 79:1078, 1982), were used for immunocytochemical demonstration of AChE-containing neurons and processes in the nucleus basalis of Meynert (nbM) and in regions of monkey brain innervated by this nucleus, especially the cerebral cortex. Macaca cynomolgus brain, fixed 24 hours in 1-2% buffered paraformaldehyde, was sectioned at 8 µm on a cryostat. The peroxidase-labeled second antibody method was used to visualize the anti-AChE antibody in the visual cortex (area 17). Diaminobenzidine-labeled fibers were present in varying densities in all cortical layers. The same pattern was encountered in each section, and no fiber staining was seen in control sections without antibody or with other antibodies. A dense, horizontally-oriented plexus was seen in layer 1. Layers 2, 3, and 4A (Brodmann) had less dense, vertically and randomly oriented fibers. A moderately dense, horizontally oriented plexus was seen in 4B. 4C had mixed long vertical and randomly oriented fiber segments in lower density. Layer 5 had a moderately dense, horizontally oriented plexus of fibers, and layer 6 had few fibers in vertical and random orientation. Deep layer 6 and superficial white matter had a dense plexus of larger fibers, continuous throughout the region, whose orientation varied in relation to sulci and gyri. The pattern of fiber staining correlates well with that revealed by AChE histochemistry, but the immunocytochemical technique was found to give much better morphological resolution. The AChE histochemical staining has been shown to be severely depleted by large lesions of the nbM. We postulate that the fiber plexus revealed in detail by immunocytochemical staining for AChE represents the cholinergic innervation of the cerebral cortex, of which at least a large proportion derives from the nbM.
(Supported by NIH NS 07179 and NSF BNS79-00035).

57.16 INTRA- AND INTERHEMISPHERIC ASYMMETRIES IN THE HUMAN BRAIN.
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A number of hemispheric asymmetries have been noted in the brains of many species (Galaburda et al., 1978; Harnad et al., 1977). In Homo sapiens, one of these asymmetries, mainly a right-frontal-left-occipital petalial pattern, i.e. a protruding of the right and left occipital poles, has been observed on CT scans (LeMay and Kido, 1978), quantified in fetal and adult brains (Weinberger et al., 1982) and has been purported to be specifically a hominid feature (Holloway & de Lacoste, 1982). Our study was undertaken to further examine this asymmetry and to determine if the protuberances of the right frontal and left occipital poles reflect underlying differences in the relative volumes of the different lobes. In addition, we examined the possibility of a sex difference within this set of cerebral relationships. Our method involved computer-assisted volumetric measurements of 3-5 mm coronal sections of adult human brains (N=18; F=7. M=11). Those brain regions selected for analysis included: Block 1 -- frontal pole to the central sulcus; Block 2 -- central sulcus to parieto-occipital sulcus; and Block 3 -- parieto-occipital sulcus to occipital pole.

Preliminary results indicate that while the R-frontal petalia is a function of a larger right frontal lobe, the L-occipital petalia reflects primarily a hemispheric difference in the volume of the parietal lobe. Furthermore, the ratio of volumes of frontal/parietal cortices is different for each hemisphere, with the left hemisphere having more parietal cortex but slightly less frontal cortex than the right hemisphere. The data also suggest that while the female brain appears to be more symmetrical in that the protuberances are less obvious at the gross macroscopic level, the left/right asymmetry in terms of the ratio of the frontal/parietal volumes is even more pronounced than in males. We believe that this is due to the finding that in females the central sulcus is frequently (6/7) more rostral on the left side than on the right side.

Our view is that volumetric measurements and analyses of the relationships between brain parts (both intra- and interhemispherical) will be critical for determining the structural bases for higher cortical functions. For example, the observation here that the left parietal lobe occupies a larger portion of its hemisphere than its right counterpart, may be related to the differential capacity of the left hemisphere to function as a substrate in the processing of language-related symbolic functions.
Supported by Biological Humanics.

57.17 INSULAR AND PARINSULAR CORTICES IN BOTTLENOSE DOLPHIN. M.S. Jacobs, P.J. Morgane and W.L. McFarland. Dept. Pathobiol. Oral Path., NYU Coll. Dentistry, New York, NY 10010; Worcester Found. Exp. Biology, Shrewsbury, MA 01545; NIH, Bethesda, MD 20014.

The mammalian neocortex has a double topologic relationship to, and is presumed to have arisen in evolution from, more primitive archicortex medially and paleocortex basolaterally. The aims of the present study are (1) to characterize the cytoarchitecture and morphometrics of the insula (Ins) and parinsular region in the dolphin brain, and (2) to compare these data with findings from a recently completed investigation of the medial juxta-archicortical limbic cortices of the cetacean brain (In Press, J. Hirnforschung).

The large, highly folded cetacean Ins is covered by a massive parinsular operculum (Operc) that is regionally organized into orbital, parietal and temporal parts. The Ins is subdivided by a central insular sulcus into a larger anterior portion which is comprised, in the dolphin, of about 10 short fingerlike radial insular gyri, and of a smaller posterior portion containing about three longer radial gyri. The neocortex covering these formations is continuous through the floor of the arcuate circular sulcus of the Ins which parallels internally the deep ectosylvian fissure present on the external brain surface that demarcates the Operc as a lobule from the rest of the hemisphere. Ins neocortex arises, through a transitional cortical plate, from the paleocortical prepyriform area (PP). The transition occupies the summit of a narrow gyrus, the transverse insular gyrus, that skirts the border between Ins and paleocortex and is demarcated internally by the rhinal sulcus. The start of Ins neocortex is signalled by the appearance of definitive layers II and V. As compared to Operc, the cortex of Ins is somewhat thinner, has a slightly lower neuronal density, and contains smaller cells. A narrow claustrum, most prominent in ventral Ins, becomes more diffuse under the transitional cortex and ends by merging with cells of PP. Differences between anterior and posterior Ins cortex, laterally, parallel those present in anterior and posterior limbic cortex, medially in the hemisphere, but are generally less fully expressed. Prominent among these differences are the organization of layer V in anterior Ins as a distinct, homogeneous cell band and in posterior Ins as cell nests which, in combination with layer VI cells, form irregular radial arrangements. Such similarities between lateral Ins and medial limbic cortices indicate that the long environmental isolation from terrestrial mammalian lines, and the considerable hemispheric expansion and folding in whales, have not affected the fundamental expression of a double origin of the neocortex in all mammals from more primitive cortices.

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57.18 SEXUAL DIMORPHISM IN HUMAN FETAL CORPORA CALLOSA. J. Baack*, C. de Lacoste-Utamsing, and D.J. Woodward. (SPON: R. Galosy). Dept. of Cell Biology, Univ. of Texas Health Science Center, Dallas, TX 75235.

Recent findings have suggested that the adult human corpus callosum is sexually dimorphic: its cross-sectional surface area relative to brain weight is larger in females than in males and the shape and size of its splenium covaries with gender (de Lacoste-Utamsing and Holloway; Science, in press). This study was undertaken to determine if similar sex differences are present in human fetal callosa. Photographs of midsagittal sections of fetal cerebra, ranging from 26-41 gestational weeks, 200-300 mm in crown-to-rump length (CRL) and 115 to 413 g in brain weight, were obtained from the Yakovlev collection (N=38; F=18, M=20). These photographs were projected at a magnification of 5.8-9.5X and were used for computer-assisted planimetric measurements of the cross-sectional surface area of the callosa (ccarea) as well as for other measurements including the dorsoventral widths of the genu, body and splenium. The male and female samples were statistically well-matched for age, brain weight and CRL.

Statistically significant differences were found between the sexes in the dorsoventral width of the splenium ($t = -2.52$; $p = .017$) and in the ratio of ccarea/brain weight ($t = -2.56$; $p = .017$). As in the adult brain, the dorsoventral width of the splenium is larger in the female than in the male ((F) $\bar{X}$=3.41 mm; (M) $\bar{X}$=2.66 mm); and for a given brain weight, the male ccarea is smaller than the corresponding female area ((F) brain $\bar{X}$=260.16 g, ccarea $\bar{X}$=61.99 mm^2, (M) brain $\bar{X}$=285 g, ccarea $\bar{X}$=55.11 mm^2; ccarea/brain, (F) $\bar{X}$=.2586, (M) $\bar{X}$=.1989). A Discriminant analysis, using splenial width, ccarea/brain and brain/(ccarea/age) as the differentiating variables, classified the callosa as male or female with 85% accuracy. There appear to be no significant sex differences in the widths of the body or genu of the callosa.

In summary, the main finding of our study is that sexual dimorphism in the human corpus callosum develops prior to the 26th week of gestation, well before most myelination occurs. It is known that the most dramatic prenatal increase in ccarea as well as splenial width occurs between 18-26 weeks of gestation and that, further, the genu and body of the callosum, which are not sexually dimorphic, undergo their major prenatal development at a later stage in gestation, mainly between 38-42 weeks gestation. We hypothesize that the sex differences in the corpus callosum are mediated by hormonal or genetic factors and that their expression is manifest before the end of the 18-26 week period of rapid development. Supported by Biological Humanics. Yakovlev collection supported by Y01-NS-7-0032-02.

57.19 NEURONAL MECHANISMS IN THE GENESIS OF SPIKE AND WAVE DISCHARGES (SW) IN FELINE GENERALIZED EPILEPSY. G. Kostopoulos, M. Avoli and P. White*. Montreal Neurological Institute & Department of Neurology & Neurosurgery, McGill University, Montreal, Canada H3A 2B4

Penicillin administration in the cat (350,000 IU/kg i.m.) induces generalized epilepsy with brief periods of "absence" accommpanied by 4-5 c/sec SW. In 77 awake but painlessly immobilized cats and during the development of this type of epilepsy we studied the extracellular responses of cortical neurons (in medial suprasylvian gyrus and pericruciate area) to thalamic (specific and non-specific nuclei) and/or PT stimulation. We found: 1) a substantial (50-200%) potentiation of the biphasic response to non-specific thalamic stimuli several minutes after penicillin administration (mean 20') and always before the appearance of SW. Single (9/17 cases) as well as repetitive (12/14 cases) stimuli became more effective while the two phases of the response (initial excitation followed by inhibition) were potentiated in parallel. The changes corresponded to potentiation of EEG recruiting responses and especially of the positive phases of the latter. Responses to specific thalamus were less markedly affected. 2) the recurrent inhibition (RI) imposed on pericruciate neurons by PT stimulation remained intact (31/32 cases) at least until the time of appearance of SW (about one hour after penicillin). There was reduction of RI in some experiments where SW appeared eventually too frequently, and in half of the PSTHs (7/13) just preceding an episode of EEG tonic-clonic seizures.

These findings along with previous evidence suggest that the recently demonstrated possibility of transition from spindles to spikes of SW (Exp. Neurol. 73: 43-71) can be attributed to a potentiation of the response of cortical neurons to non-specific, spindle-inducing thalamocortical volleys. The increased cortical output thus produced may activate a feedback inhibitory mechanism which curtails this excitation and prevails for the period of the wave component of SW. We also show that intracortical RI is not antagonized by these doses of penicillin and can at least participate in this mechanism. However additional mechanisms may contribute as well. The transition from SW to generalized tonic-clonic episodes in this model may coincide with a breakdown of RI.

57.20 RESPONSE DECREMENT IN EVOKED POTENTIALS RECORDED FROM THE *IN VITRO* NEOCORTICAL SLICE.

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The dual process theory of habituation (Groves and Thompson, *Psych. Rev.*, 1970, 77, 409-450) suggests that different anatomical structures might be involved in different aspect of the phenomenon. The present investigation examined habituation and sensitization in the *in vitro* neocortical slice (Shaw and Teyler, *Brain Res.*, 1982, in press). Briefly, 400u thick coronal sections were obtained from the dorsomedial parietal and occipital area of rat neocortex. Responses to stimulation of the underlying white matter were recorded from layers II and IV. In addition, responses to stimulation of layer IV were recorded from layer II and from an adjacent area of layer IV.

In each case input/output functions were determined for each pathway and stimulation was set at 50% of maximum response. A train of 10 stimuli were delivered at a rate of 1/sec., followed by a single recovery stimulus applied 10 sec. after the end of each train. Five of the 9 parameters of habituation outline by (Thompson and Spencer, *Psych Rev.*, 1966, 73, 16-43) were examined. Habituation, Spontaneous Recovery, the effects of Intensity, the effects of Frequency, and Sensitization.

Results indicated that of the four neocortical pathways examined, only one (the layer II response to white matter stimulation) displayed significant response decrement to repeated afferent stimulation. The time course of the decrement and its spontaneous recovery was similar to that observed in another CNS structure, the dentate gyrus of the hippocampus. The effects of frequency and intensity were as predicted by theory and as observed in the dentate gyrus. Significantly, however, this neocortical pathway failed to demonstrate Sensitization. These results support the dual-process theory of habituation and have implications for cortical functioning. (supported by NSF and NIH).

58.1 HYPOTHALAMIC SEXUAL DIMORPHISMS IN FOUR RODENT SPECIES. W. Byne*, R. Bleier, and I. Siggelkow*. Neuroscience Training Program and Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

An extensive configuration of cells lying in the medial preoptic-anterior hypothalamic region of the guinea pig, rat, hamster and mouse exhibits sex differences in the pattern of cell density and distribution at most levels. This configuration which we call the sexually dimorphic nuclear complex of the medial preoptic-anterior hypothalamic area (SDNC-MPAH) exhibits considerable cross-species variation although the most conspicious sex differences are seen in the medial preoptic nucleus (MP) in all 4 species and in the anterior hypothalamic nucleus (AH) of all except the mouse. The sex difference associated with MP is of particular interest since lesions confined to this nucleus block spontaneous ovulation in the rat (Terasawa et al., 1980) and guinea pig (Terasawa et al., 1982).

For this study 5 guinea pig brains of each sex and 3 rat, mouse and hamster brains of each sex were embedded in celloidin, sectioned at 30 µm, stained with thionin and coded so that sex of the preparation was not known to the investigators. The investigators independently identified the sex of all preparations by visual inspection at low magnification.

In all species except the hamster, MP is well developed in both sexes as a singular triangular configuration of cells situated just caudal to the lamina terminalis (LT). In the female hamster, a configuration which we feel corresponds to MP in the other species begins just caudal to LT, continues caudally and divides into several densely staining subgroups. In the male, MP begins as in the female but ends a few sections caudally by merging with a more diffuse cell grouping. More caudally still, this grouping of cells appears to increase in density and closely resembles the female configuration at the same level. In this study, we designated only the more anterior cell density described above as MP in the male hamster. The volume occupied by MP was determined with computer assistance for all preparations. In all species MP occupied a greater volume in females than males.

Within AH of the rat and guinea pig, cells are concentrated more medially in the female than the male. Additionally, within AH an approximately round configuration of increased cell density can be distinguished in the male and female rat and hamster but only in the male guinea pig. This configuration corresponds to the sexually dimorphic nucleus of the preoptic area (SDN) previously described in the rat (Gorski et al., 1978). We found the SDN to occupy a greater volume in the male than the female rat, corroborating the reports of other laboratories.

58.2 AN EM AUTORADIOGRAPHIC STUDY OF THE MODE OF TERMINATION OF THE HYPOTHALAMIC PROJECTION TO THE DENTATE GYRUS OF THE RAT. Judith A. Dent*, Brent B. Stanfield and W.Maxwell Cowan. The Salk Institute, La Jolla, CA 92138 and The Clayton Foundation for Research-California Division.

Previous light microscopic studies have demonstrated the existence of a projection which arises in the supramamillary region of the hypothalamus and terminates in a narrow zone within the dentate gyrus. Since this projection is concentrated over the superficial part of the granule cell layer and the deepest portion of the overlying molecular layer it has been suggested that these fibers synapse upon either the granule cell somata or the proximal parts of their dendritic shafts. In order to clarify the mode of termination of this projection we have injected high specific activity tritiated proline (proline [L-2,3,4,5 -^{3}H] specific activity 139.1 Ci/mmol) into the supramamillary region in a series of twelve young adult rats. After a 4 or 7 day survival period, the animals were perfused with a buffered solution of 2% paraformaldehyde/2% glutaraldehyde, the brains were removed, and slices from the rostral dentate gyrus were processed for EM autoradiography.

In accordance with our previous light microscopic findings, in our EM autoradiographs the hypothalamo-dentate projection has been found to terminate in a narrow zone that extends from about the middle of the granule cell layer to the inner one-fifth or so of the molecular layer. Within this zone more than 80% of the silver grains seen were associated with vesicle-containing profiles, and in all of these, the vesicles were clear-centered and distinctly round in shape. Where the membrane specializations of the labeled terminals were clearly recognizable, they were invariably asymmetric. The majority of the labeled synapses could be identified as terminating on large dendritic shafts which arose from granule cells, but a smaller number of axo-somatic and axo-spinous synapses was also seen. The labeled axo-somatic and axo-spinous synapses were also associated with clear, rounded, vesicles and had asymmetric membrane specializations.

That the hypothalamic terminals have the morphological features commonly associated with an excitatory input is somewhat surprising in view of previous electrophysiological observations which have been interpreted as evidence that this projection provides a monosynaptic inhibitory input to the dentate granule cells. Thus, while our study has clarified the mode of termination of the hypothalamo-dentate fibers, their mode of action upon the granule cells remains to be determined.

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58.3 THE PARAVENTRICULAR NUCLEUS OF THE RABBIT HYPOTHALAMUS: A GOLGI STUDY. S.D. Farber* and D.L. Felten. Dept. of Anat., Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

Neonatal (3 week old) and adult rabbit brains were prepared for Golgi-Cox impregnation to determine the cytoarchitectural characteristics of the major cell types in the paraventricular nucleus (PVN). In some brains, from 100 to 200 neurons were sufficiently impregnated to permit excellent visualization and quantitation. Three major cell types were found in the PVN: (1) large multipolar neurons; (2) large bipolar neurons; and (3) small interneurons. The large multipolar neurons were the most abundant cell type (51.4% in neonates, 45.2% in adults), and were densely concentrated in the lateral portion of the PVN, and in the ventral portion of the rostral PVN. These neurons ranged from 14.5 to 29 ums in diameter in neonates and from 14.5 to 37.7 ums in adults. Primary dendrites branched in random directions, and gave rise to secondary dendrites close to the soma. Tertiary branching was common in adults. The dendrites and somas of multipolar PVN neurons possessed spiny, irregular surfaces in neonates, but appeared vesicular and smooth in adults, with a striking reduction of spines. The large bipolar neurons represented 19.4% of the PVN population in neonates and 22.2% in adults. These bipolar neurons were concentrated in the medial portion of the PVN and were particularly abundant in the dorsomedial portion of the caudal PVN. The long axis of the bipolar cells ranged from 17.4 to 31.9 ums in neonates and 17.4 to 37.7 ums in adults. In neonates, the major orientation of the bipolar cell axes was horizontal, but changed to an oblique orientation in adults. Frequent secondary and tertiary dendritic branching was noted. In neonates, the dendrites and somas possessed numerous spines, but in adults the dendritic spines were greatly reduced in number in vesicular dendritic profiles, while some somatic spines persisted. The small interneurons were irregular in shape, possessed sparse cytoplasm, and gave rise to numerous primary and secondary dendrites. The interneurons ranged from 5.8 to 17.4 ums in neonates and 5.8 to 20.3 ums in adults. They were scattered throughout the PVN, and represented 29.2% of the neuronal population in neonates and 32.5% in adults. The dendrites were highly spiny, with a barbed-wire appearance in adults and retained their spiny appearance in adults. The magnocellular neurons are regionally distributed by somatic shape, and their dendrites show increased branching with reduced presence of spines during maturation. The spiny interneurons represent a significant portion of neurons in PVN and may represent an important link in afferent connectivity with the magnocellular neurons, and may play a role in autonomic regulation as well as neurosecretory regulation.

58.4 The Primate Medial Mamillary Nuclei: An Evolutionary Perspective of Morphometric Data. E. Armstrong* (Spon: D.E. Smith). Dept. Anatomy, L.S.U. Med. Ctr., New Orleans, La. 70112.

Earlier work has shown that parts of the limbic system are relatively enlarged in the human brain. The data presented here examine some quantitative and allometric relationships of the medial mamillary nuclei (MMN) among primates. The morphometric data are based on measurements taken from Nissl stained serially sectioned brains of 19 primate genera. Nuclear volumes were determined by measuring the areas of cytoarchitecturally defined nuclei and estimates of neuronal density came from systematic sampling of neuronal nucleoli throughout the nuclei.

In addition to the quantitative data, a major cytoarchitectural difference was noted. Prosimians, including Tarsius, differ from the anthropoid primates in merging the left and right MMN across the midline. This does not appear to be a function of brain size since large brained prosimians (L.catta brain wt. = 22.9g. and L. mongoz brain wt. = 21.8g.) showed this characteristic, while New World monkeys whose brain weights overlap those of prosimians (S. tamarin brain wt. = 8.1g and Aotus brain wt. = 15.8g) have cytoarchitecturally distinct bilateral nuclei.

Morphometric and allometric analyses show that the differences in MMN volume and numbers of neurons covary both with body size and, more strongly, with brain weight. As expected the size of MMN is also very highly correlated with that of the thalamic anterior complex (AP). The prosimian MMN compose a slightly higher proportion of the brain and AP than they do in anthropoids. However, when MMN volume is studied as a function of either brain size or AP volume, the rate of MMN volume increase in prosimians is somewhat lower than that among anthropoids. The anthropoid slopes relating MMN volume to brain weight and AP volumes are both steeper and also come close to predicting the observed human values. The quantitative and cytoarchitectural differences between Prosimii and Anthropoidea suggest that following the separation of the two major primate infraorders, aspects of MMN evolved along divergent paths.

While the volume of the anterior thalamic complex is much larger than MMN, the numbers of neurons found in AP is not much greater than that of MMN. The significance of volumetric increases which do not entail increases in the numbers of neurons is a puzzle as yet, but it might suggest an increase in dendritic arborization and integrative functions.

By examining the volume, neuronal density and cytoarchitectural structure of the primate CNS, it is possible to gain insight into the evolutionary process which formed the human brain.

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58.5 AN AUTORADIOGRAPHIC STUDY OF THE EFFERENT CONNECTIONS OF THE MAMMILLARY NUCLEI OF THE GUINEA PIG. C. L. Shen. Inst. of Neuroscience, Natl Yang Ming Med. Col., Taipei, Taiwan, Republic of China.

The efferent projections were traced after injecting tritiated amino acid into the medial mammillary nucleus (MMN) and lateral mammillary nucleus (LMN) of the guinea pig. The animals were sacrificed at 52 to 103 hours and processed for autoradiography. The major efferent was the principal mammillary tract. After a short coursing from the mammillary nuclei, this bundle divided into two distinct tracts, an anterodorsal projection-mammillothalamic tract (MTH) and a caudal projection-mammillotegmental tract (MTE), and a few dorsal projecting fibers. The MTH ran anterodorsal direction via the posterior hypothalamus and ventromedial thalamic nuclei. It terminated in the anterior medial, anterior dorsal and anterior ventral thalamic nuclei. The MTE projected caudally. After running into the midbrain, this tract spreaded into a less compact fiber system. Most of fibers in the MTE turn dorsocaudally and terminated in the ventral and dorsal tegmental nuclei. The other descending fibers ran via the pontine tegmentum and terminated in the pontine nucleus, pontine tegmental nucleus and reticular formation. The dorsal projection from the principal mammillary tract projected dorsally along the posterior edge of the thalamus. These fibers terminated in the central gray of the brain stem down to the area rostral to the hypoglossal nucleus.This central gray projection was enhanced by fibers from the MTE. Some MMN diffused fibers projected dorsally through the posterior hypothalamus and terminated in the midline nuclei of the thalamus, Rostral projections from the MMN projected anteriorly via both the medial hypothalamus-preoptic area-diagonal band of Broca, and medial forebrain bundle. They terminated in the medial and lateral septa. These MMN projections were bilateral.

LMN sent fibers joined the MTH and MTE. Except anterior medial nucleus of the thalamus, the LMN efferents terminated bilaterally in the anterior ventral and anterior dorsal nuclei of the thalamus, dorsal and ventral tegmental nuclei, pontine nuclei and reticular formation.

No labeled fibers were found in the fornix. Such result demonstrated that tritiated amino acid could not be taken by axonal terminals. (Supported by NSC (ROC) grant 70-0412-B010-21).

58.6 A HORSERADISH PEROXIDASE STUDY OF THE AFFERENTS TO THE ENTORHINAL CORTEX OF THE MONKEY. R.Insausti*#, D.G. Amaral and W.M. Cowan. The Salk Institute, P.O. Box 85800, San Diego, CA. 92138 and Dept. of Anatomy, Univ. of Navarra (SPAIN)#

The entorhinal cortex (EC) is known to be the major link between the hippocampal formation and several areas of the cerebral cortex. Yet despite this, our knowledge of the organization of the afferents to the EC in the primate is still rather fragmentary. We have begun a systematic analysis of these connections by making small (100 nl) injections of a 1% solution of wheat germ agglutinin-conjugated horseradish peroxidase, into both the lateral (LEC) and medial (MEC) divisions of the EC, and into the adjoining periamygdaloid and perirhinal cortices in a series of cynomolgus monkeys. After survival periods of 48 hrs the brains were processed according to the TMB method of Mesulam.

The entorhinal cortex has been found to receive inputs from a wide variety of cortical and subcortical structures including: (i) The hippocampal formation: here cells in the regio superior, the subiculum, and the pre-and parasubiculum have been found to project to the EC; those in the presubiculum project exclusively to the MEC both ipsilaterally and contralaterally. (ii) The contralateral EC: the MEC (but not the LEC) receives a homotopic commissural projection. (iii) The temporal neocortex: these afferents originate in the perirhinal cortex (area 35), areas TF-TH, TG, and the anterior portion of TE. There is also a relatively strong projection from the dorsal bank of the superior temporal sulcus, to the MEC. The LEC receives an input from the dorsal surface of the superior temporal gyrus and the ventral insular cortex. (iv) Frontal cortex: several orbitofrontal cortical regions (areas 14,13,12 of Walker) send fibers to the EC, but fewer labeled cells are also found in the dorsolateral prefrontal cortex (areas 9,10,46) after injections into the EC. The entire rostrocaudal extent of the cingulate cortex projects to the EC; caudally this projection continues into the retrosplenial cortex and the anterior portions of prestriate cortex. (v) Amygdala: the lateral and basolateral nuclei of the amygdala and the periamygdaloid cortex project to the EC. (vi) Subcortical structures: subcortical projections to the EC originate in the medial septal nucleus, the nucleus of the diagonal band, the substantia innominata, the thalamus (paraventricular and paracentral nucleus, nucleus reuniens, and the medial pulvinar) the hypothalamus (mainly from the supramamillary and tuberomamillary nuclei) and brain stem (periaqueductal gray matter, dorsal and central superior nuclei of the raphe complex, and the locus coeruleus).

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58.7 EARLY DEVELOPMENT OF THE SUPRACHIASMATIC NUCLEUS OF THE RAT: AN ULTRASTRUCTURAL STUDY. M. F. Bernstein and R. Y. Moore, Department of Neurology, SUNY at Stony Brook, N. Y. 11794.

The fine structure of early development of the suprachiasmatic nucleus (SCN) was analyzed in the rat from embryonic day 19 (E19) through postnatal day 4 (P4). The occurrence of cell death, maturation of the SCN-optic chiasm (OC) relationship, development of the neuropil and synaptogenesis was studied. Three animals from E19, E21, E22, P1 and P4 were intracardially perfused with buffered aldehydes and prepared for electron microscopy by conventional methods. Coronal sections taken from two middle levels of the SCN were examined.

Degenerating perikarya are evident throughout the SCN from E21 through P2 with maximal numbers occurring between E22 and P2. At E19 and E21 the neuropil is dominated by dendrites and dendritic growth cones. Few synapses are present prenatally. In dorsal areas maturation of the neuropil, with an increase in the number of axons and axonal growth cones and the genesis of synapses, proceeds steadily from E19 to P2 when an increase in the rate of synaptogenesis is observed. This rate is maintained through P4. The ventrolateral SCN contains few synapses before P2. The development of the SCN-OC relationship with the entry of axons from the OC and a concomitant invasion of the OC by dendrites and scattered perikarya occurs on P1-P2. The SCN-OC interface is characterized at this time by a complex matrix of axons, growth cones, dendritic profiles and immature synapses. With the maturation of the SCN-OC relationship, a rapid synaptogenesis begins which results in almost twice the number of synapses in this area as are present in other areas of the SCN at P4. The ventromedial area of the SCN is the slowest to demonstrate maturational changes, rarely containing synapses until P2. However, by P4, the neuropil of the ventromedial SCN is indistinguishable from other areas of the SCN and contains as many synapses as the dorsal areas.

In summary, the development in dorsal areas of the SCN precedes that in ventral areas. Afferents from the OC enter the ventral region at P1-P2, probably contributing to the marked increase in the rate of synaptogenesis evident throughout the SCN. Consequently, the development of the SCN neuropil should be viewed as a largely early postnatal process.

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58.8 DEMONSTRATION OF HIPPOCAMPAL FORMATION AXONS IN THE RAT BY INTRACELLULAR INJECTION OF HORSERADISH PEROXIDASE. David M. Finch, Nancy L. Nowlin* and Thomas L. Babb. Reed Neurological Research Center and Brain Research Institute, Univ. of California, Los Angeles, CA 90024.

Hippocampal formation neurons of rats were injected intracellularly with horseradish peroxidase either by iontophoresis or pressure. After postinjection survival times of 8-20 hours the animals were re-anesthetized and perfused. Their brains were extracted, sectioned at 200 μm in the horizontal plane, and reacted according to the TMB protocol of Mesulam (J. Histochem. Cytochem., 26 (1978) 106-117). In favorable experiments axons could be traced 1-4 mm through serial sections.

One CA1 cell with physiological properties associated with inhibitory interneurons (Schwartzkroin and Mathers, Brain Research, 157 (1978) 1-10) was recovered. Although classic basket endings were not seen, it was most similar to the poligonal basket cells described by Lorente de Nó.

Seven CA3 pyramidal neurons were recovered, all of which showed 1 or 2 axonal processes that could be traced rostrally through the alveus to the fimbria. Schaffer collaterals to CA1 could be seen in four of the neurons.

Results from CA1 pyramidal neurons have been previously described (Finch and Babb, Brain Research, 214 (1981) 405-410). Six more recent cases were generally similar except that most of the cells (5 out of 6) showed both rostrally and caudally projecting axonal branches. In three instances a caudally directed branch could be traced in the alveus to the level of the subiculum.

Ten subicular pyramids were recovered. Three showed axons which could be traced only rostrally, toward the fimbria. Four showed axons which could be traced only caudally and/or laterally. In one of these cells an axonal branch could be traced caudally through white matter of the angular bundle to entorhinal cortex. The remaining 3 subicular neurons showed major branching projections, with both rostrally and caudally directed axonal collaterals. One of these cells showed projections to three distinct brain areas: (a) 1 rostrally directed axonal branch via the alveus to the fimbria; (b) 2 caudally directed branches via the angular bundle to entorhinal cortex; and (c) 1 medially and dorsally directed branch to cingulate cortex. The results further emphasize the subiculum's role as the recipient of input from the hippocampus proper and as a major efferent structure of the hippocampal formation.

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58.9 A SPECIFIC INTERNEURON FORMING SYNAPSES EXCLUSIVELY WITH THE AXON INITIAL SEGMENT OF PYRAMIDAL CELLS IN MONKEY HIPPOCAMPUS.
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We have identified in Golgi preparations of monkey hippocampus a type of interneuron, whose axon profusely arborizes in the pyramidal layer. The perikaryon, located in the upper portion of pyramidal layer, is of medium size, with radially oriented smooth dendrites. The outstanding feature of this cell is that its axon forms characteristic rows of boutons, 40-80 µm long, oriented parallel to the course of the axons of pyramidal cells. Several thin axon collaterals may contribute to a single terminal segment.

Light microscopy reconstruction of an identified interneuron has revealed more than 200 terminal segments, spread in an area of 300 µm in diameter. The synaptic relationship of terminal boutons have been established using a combined Golgi - EM technique. Two complete cells and isolated axonal plexuses have been traced in serial thin sections. Each row of boutons is found in synaptic association exclusively with the axon initial segment of pyramidal cells, therefore we call this neuron axo-axonic cell in analogy with neurocortical neurons (Somogyi, P., Brain Res., 136: 345, 1977). The impregnated boutons form symmetrical synapses and contain pleomorphic vesicles.

The strategic location of terminal rows and their distributions over a large populations of pyramidal cells, could account for neuronal synchronisation during hippocampal events. The interneuron, if involved in the last link of a recurrent inhibitory pathway, might contribute to generation of rhytmic activity. Moreover, as the hippocampus is a brain area particularly prone to seizures, it is feasible that epilepsy is the result of some alterations of the normal pattern of control exerted by this category of interneuron.

58.10 INTERNEURON MORPHOLOGY IN THE CA1 REGION OF RABBIT HIPPOCAMPUS.
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Interneurons are thought to play an important role in the function of hippocampus. With intracellular dye injections, electrophysiological characteristics of identified interneurons have been described; simultaneous intracellular recordings from interneuron-pyramidal cell pairs have confirmed some of our assumptions about inhibitory interneuron connectivity. We present here electron microscopic data describing CA1 hippocampal interneurons in rabbit. The primary interneuron population we have encountered has somata located in stratum oriens near the pyramidale border (within 70 µ), as shown with intracellular injections of horseradish peroxidase and Lucifer yellow as well as in Golgi preparations. They are large (~50 µ diameter), have roundish cell bodies and beaded dendrites projecting both apically and basally.

At the EM level, these cells have nuclei with complex, infolding membrane which are quite distinctive. As compared to the somata of pyramidal cells, the interneuron nucleus takes up a smaller proportion of the soma; the interneuron also has a denser somatic cytoplasm, more ER and polyribosomes, and is covered with many synapses (mostly asymmetric). These somata give rise to dendrites projecting through pyramidale into radiatum, and to (and sometimes into) the alveus. The dendrites are of large diameter (although smaller than primary pyramidal cell dendrites) and can be traced for substantial distances without marked tapering. The dendritic cytoplasm is denser than that of pyramidal cells, and contains a large number of mitochondria, which are often concentrated in regularly occurring dendritic swellings. The dendrites have no spines, but are densely covered with asymmetric synapses directly on the shaft membrane.

Preliminary immunohistochemistry on rabbit hippocampus (with A. Hendrickson) has revealed a multitude of other neuronal cell types which are likely to be interneurons. Cells containing GAD are quite numerous in stratum oriens; this population probably overlaps with the cell population described above, and with the functional inhibitory interneuron population. In addition, there are distinctive neurons in pyramidale which stain for enkephalin, and neurons at the oriens-alveus border which stain for somatostatin; both cell types send processes into stratum radiatum, and may be functionally excitatory to pyramidal cells. These data illustrate the richness of hippocampal interneuron variety and indicate the need for more complex, realistic models of hippocampus.

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58.11 TRANSNUCLEAR TRANSPORT OF HRP: LIGHT AND ELECTRON MICROSCOPIC STUDIES OF MAMMILLOTHALAMIC AND MAMMILLOTEGMENTAL PROJECTIONS.
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Numerous studies have explored the connectivity of the mammillary nuclei but because of their complexity and divergent axon collaterals many details of the organization of their projections remain unclear. In the present study, the collateral projections of the mammillary nuclei have been investigated with the aid of the axonal transport of horseradish peroxidase (HRP) as first demonstrated by de Olmos and Heimer (Neurosci. Lett., 6:107, 1977). In 35 Wistar rats, 0.01-0.03 µl of a 5% aqueous solution of lectin-HRP (Sigma) were injected into the anterior thalamus or into the tegmentum. After 2-day survival periods the rats were perfused and their brains were processed for light microscopy using tetramethyl benzidine (Mesulam, M.-M., J. Histochem. Cytochem., 216:106, 1978) or for electron microscopy using diaminobenzidine (Adams, J.C., J. Histochem. Cytochem., 29:775, 1981).

Large injections of HRP into the anterior thalamus gave rise to anterograde labeling in the contralateral anterodorsal thalamic nucleus and retrograde labeling throughout each of the divisions of the ipsilateral mammillary nuclei as well as in the contralateral lateral mammillary nucleus. Anterograde labeling in the terminal fields of descending collaterals of mammillary nuclei somata was observed in the dorsal tegmental nucleus (DTN) bilaterally. In the ventral tegmental nucleus (VTN) very heavy anterograde labeling was observed, primarily ipsilaterally. Anterograde labeling was also observed in the tegmental reticular nucleus (TRN) bilaterally. Small injections of HRP into the anterior thalamic nuclei gave rise to specific patterns of labeling in the mammillary nuclei and tegmentum. Electron microscopy of the VTN demonstrated that descending axon collaterals of mammillothalamic neurons formed axosomatic and axodendritic synapses. Most labeled terminals in the VTN contained round vesicles and made asymmetrical synaptic contact with the plasmalemma.

After injections of HRP into the tegmentum, anterograde and retrograde labeling were observed in the ipsilateral medial and lateral mammillary nuclei. Anterograde labeling of axon collaterals was present bilaterally in the anterodorsal nucleus and ipsilaterally in the anteroventral and anteromedial nuclei of the thalamus.

The present results indicate that each of the major divisions of the mammillary nuclei projects to both the thalamus and tegmentum. The lateral mammillary nucleus appears to project mainly to the DTN while the medial mammillary nuclei project mainly to the VTN and TRN. Supported by MRC of Canada, the Killam Foundation and the Dalhousie Medical Research Foundation.

58.12 HYPOTHALAMIC AFFERENTS TO THE DORSAL DENTATE GYRUS CONTAIN ACETYLCHOLINESTERASE.
J-C. Lacaille*, C. W. Harley and M. Galway* (SPON: C. Malsbury). Psychology Department, Memorial University of Nfld., St. John's, Nfld. A1B 3X9

Glass micropipettes with tip diameters of 100µ were filled with powdered Evans blue or HRP and implanted in the dorsal dentate gyrus of anesthetized Sprague-Dawley rats (n=30). Pipettes were left in place for 1-4 hr. or cemented in place until perfusion 1-3 days following implantation. Evans blue animals were perfused with .9% saline followed by 10% formalin. Following cryostat sectioning at 50µ the brains were examined for Evans blue in a Zeiss Epi-fluorescence microscope and labelled hypothalamic cells were photographed together with the implant site. Sections were then reacted for AChE in Shute-Lewis medium and the esterasic deposits visualized with potassium ferricyanide. Combined AChE and HRP tracing followed Mesulam's protocol (J. Histochem. Cytochem. 24:1281-6, 1976).

Evan's blue tracing appeared to be considerably more sensitive than the HRP method in that larger numbers of well-filled cells were seen in the Evans blue experiments. For example in one serially sectioned brain with a restricted injection site more than 500 Evans blue cells were counted in the hypothalamus ipsilateral to the injection site. Moreover although identifying the co-occurence of Evans blue and AChE reaction product involved matching sections and photographs after the esterase reaction the identification of esterasic labelled cells was accomplished much more easily than the identification of double reaction product in the HRP reacted tissue.

Labelled cells were found in the caudolateral hypothalamus. They extended from the first appearance of the mammillary recess to the caudal portion of the mammillary bodies with no marked rostrocaudal discontinuities. The cells initially exhibit a close relationship with the mammilothalamic tract and then fan laterally and ventrally through the caudolateral hypothalamus. In the mammillary region labelled cells were always lateral to the medial margin of the mammillothalamic tract and co-extensive with the large celled portion of the supramammilary area.

Virtually all of the labelled cells could be positively identified as containing AChE. In sample sections the %age of cells positively identified as esterasic varied from 80% to 100%. The cells'esterasic reaction was moderate and contrasted with nearby cells of intense reactivity. It would appear that, like several other identified afferents to the dentate gyrus, the extensive hypothalamic input is cholinesterasic.

58.13 OVERLAPPING CHOLINERGIC AND NONCHOLINERGIC SEPTOHIPPOCAMPAL PROJECTIONS. R. H. Baisden, M. L. Woodruff and D. B. Hoover. Depts. of Anatomy and Pharmacology, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, TN 37614.

It is well known that the medial septal and diagonal band regions supply cholinergic projections to Ammon's horn. However, whether or not neurons located in these areas also supply noncholinergic projections to hippocampus requires further evidence (Lynch et al., CIBA Symposium 58, 1978). To supply such evidence a technique which combines acetylcholinesterase (AChE) staining with the retrograde transport of HRP was applied in the present experiments. In addition, in order to determine the geometry of the cells supplying the projections, neurons impregnated by the Golgi procedure were compared to HRP-containing cells. Fifteen rabbits were given 0.5µl injections of 30 to 60% HRP (Boehringer, Grade I) into the dorsal hippocampus from its septal end caudally to the point of flexure. The brains of these animals were subsequently prepared for simultaneous visualization of HRP reaction product and AChE according to the procedure of Mesulam (J. Histochem. Cytochem., 24:1281, 1976). The brains of 3 additional rabbits were prepared with the Golgi-Kopsch technique. Inspection of Golgi-impregnated neurons within the diagonal band and medial septal area indicated that these cells were fusiform and multipolar isodendritic neurons. Fusiform cells were found along the midline of the medial septal area and at the base of the brain in the diagonal band. The multipolar cells were found in more lateral and dorsal positions. Comparison of these neurons to neurons observed in the double-labeled brains indicated that both fusiform and multipolar cells were labeled with HRP and AChE. Many of the cells of both types were labeled with HRP, but did not stain for AChE. Double-labeled cells were also found. There was no systematic difference in location of the differently labeled cells within the areas studied.

These observations indicate that many of the cells in the medial septal nuclei and diagonal band which project to the hippocampus are not cholinergic. In addition, although there is a differential distribution of fusiform and multipolar cells within these areas, this difference does not appear to relate to whether or not these cells project to the hippocampus, or, when they do project to the hippocampus, to whether or not they contain AChE.

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58.14 EFFERENT PROJECTIONS OF THE NUCLEUS OF THE DIAGONAL BAND OF BROCA TO THE OLFACTORY BULB AND MIDLINE CORTEX. K. Sripanidkulchai and J.M. Wyss. Dept. of Anatomy, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

The nucleus of the diagonal band of Broca is a basal forebrain structure divisible into a horizontal limb (HNDB) and a vertical limb (VNDB), with the latter further subdivided into a dorsal (VNDBd) and ventral (VNDBv) part (Price and Powell, '70; de Olmos et al., '78). The efferent projections from this nucleus are widespread, with the horizontal limb projecting primarily to the olfactory bulb and the vertical limb projecting primarily to the hippocampal formation. The projection to neocortex remains controversial. In order to determine the organization of the diagonal band projection to the olfactory bulb and cingulate cortex, 40 albino rats were each first injected with a single, small amount (50-150nl) of 2% True Blue [Illing, West Germany and later injected with a similar amount of 1% Nuclear Yellow [Loewy, West Germany]. Each injection was stereotaxically placed (via 10µl syringe or a 30µm tip glass pipet) into the main olfactory bulb (MOB) or one of the subareas of the cingulate cortex. After appropriate survival times the animals were reanesthetized and transcardially perfused with normal saline (100ml) followed by 10% formalin in 0.1M phosphate buffer at pH 7.4 (300ml). The brains were removed, soaked in a formalin-sucrose solution overnight and 30µm frontal sections were cut on the freezing microtome. Subsequently the tissue was mounted on a clean slide and observed under a Leitz A cube filter system. In agreement with past studies, injection of the MOB resulted ipsilateral labeling of neurons in anterior olfactory nucleus, primary olfactory cortex, entorhinal cortex and the VNDB. Some neurons in the VNDBv adjacent to the VNDB were also labeled. Injections into the various subareas of cingulate cortex resulted in the labeling of neurons within the VNDBv with lesser labeling in the VNDBd. No labeled cells were observed in the HNDB. Injections of the cingulate cortex and medial olfactory bulb with different dyes, resulted in labeled cells which overlapped in the VNDB but no double labeling was observed. Posterior versus anterior cingulate injections likewise result in the close positioning of labeled neurons but little double labeling. Therefore, the VNDB axons apparently do not collateralize as much as might be predicted from their total number versus their widespread distribution.

*Price JL and TPS Powell, 1970, J. Anatomy (London) 107:215-237.
de Olmos JS, H Hardy and L Heimer, 1978, J. Comp. Neur. 181:213-244.

58.15 SOME OBSERVATIONS ON FUSIFORM NEURONS IN STRATUM ORIENS OF THE HIPPOCAMPAL FORMATION OF THE RHESUS MONKEY. D.L. ROSENE AND N.J. ROY*. Dept. of Anatomy, Boston University School of Med., Boston, MA. 02118.

The pyramidal cells of the mammalian hippocampal formation have been investigated using a variety of anatomical techniques. Such studies have identified the pyramidal cells as the projection neurons of the hippocampus and differences in pyramidal cell morphology have provided the basis for most architectonic subdivisions of the hippocampus. On the other hand, various types of non-pyramidal cells have received less attention. In rodents, physiological investigations have called attention to the possible role of non-pyramids as interneurons and some non-pyramids have recently been characterized by immunocytochemical studies, pointing out the potential importance of non-pyramidal cells in the organization of the hippocampal formation.

Virtually nothing is known about non-pyramidal cells in the rhesus monkey. In the course of our investigations of the monkey hippocampal formation we have initiated a Golgi analysis of the hippocampal formation. Among the non-pyramidal cells that we have observed is a fusiform neuron located in stratum oriens, most frequently in the CA1 subfield near CA2. These spindle-shaped neurons have a bipolar dendritic tree with sparsely-spinous dendrites extending up to 1000 microns from the soma within the stratum oriens in a plane transverse to the longitudinal axis of the hippocampus. Occassional branches of these main dendrites ascend into stratum pyramidale. When present, axons either enter the alveus or ascend into the stratum pyramidale coursing in the longitudinal plane.

While it has not been possible in our Golgi preparations to follow these axons to their termination, morphologically similar neurons have been labeled by injections of the retrograde tracer, horseradish peroxidase (HRP). Injections of HRP into the hippocampal formation labelled fusiform neurons in stratum oriens of CA1 both rostral and caudal to the injection site. In addition an injection of HRP into the septal area and the diagonal band also labelled fusiform neurons in stratum oriens of CA1. It is clear that as a class these fusiform neurons have both local-circuit termination within the hippocampus as well as extrinsic projections that reach the septal area. It remains for retrograde double-labelling experiments to determine if individual neurons project both intrinsically and extrinsically.

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58.16 SUBCORTICAL EFFERENTS OF THE AMYGDALOID COMPLEX IN THE MONKEY. K.C. Kosel and D.L. Rosene (SPON: D. Pandya). Boston Univ. School of Medicine, Dept. of Anatomy, Boston, MA. 02118

The amygdaloid complex in the monkey consists of a number of cytoarchitecturally well defined nuclei which give rise to various intrinsic and extrinsic projections to both cortical and subcortical areas. Despite the recent demonstrations of various amygdalofugal projections in the monkey, little conclusive evidence exists with regard to the specificity of these projections or their precise sites of origin. In an attempt to address some of these problems, discrete injections of tritiated amino acids were made into various nuclei within the amygdaloid complex.

Eight monkeys received small injections of a mixture of ^{3}H-labeled amino acids (10-30 µCi in 0.1 to 0.3 µl). The injections were made either stereotactically or visually via an inferior temporal approach. The animals survived 5 to 7 days and were perfused and processed for autoradiography in a conventional manner.

The results reveal that injections confined to the cortical and medial nuclei give rise to termination over the ventromedial hypothalamic nucleus, while injections involving either the mediobasal or laterobasal nuclei resulted in terminal labeling in discrete regions of the lateral hypothalamic area. In the thalamus, terminal label was restricted to the mediodorsal nucleus, and only occurred following injections in the mediobasal and laterobasal nuclei. Terminal labeling was also observed over the bed nucleus of the stria terminalis and the horizontal limb of the nucleus of the diagonal band following injections in any of the amygdaloid nuclei except for the lateral, whereas only injections which involved the deep nuclei (laterobasal, mediobasal, accessory basal and central) resulted in extensive labeling throughout Nucleus Basalis of Meynert. Finally, a sizable projection was found from the cortical and medial nuclei to the ventral tegmental area.

These findings are of interest in view of the fact that the amygdala is functionally related to various behavioral and autonomic responses associated with these subcortical areas. It is apparent from the results of this study that a number of the efferent projections previously described, arise from specific nuclear groups within the amygdala and that the projections described provide an anatomical basis for specific interactions between the amygdala and these subcortical areas.

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58.17 HYPOTHALAMIC PROJECTIONS TO THE SPINAL CORD IN THE HAMSTER. L.L. Don Carlos* and J.A. Finkelstein, (Spon.: M. Schechter). Northeastern Ohio Univs. College of Medicine, Rootstown, OH 44272.

Localization of hypothalamic efferents to the spinal cord in hamsters was determined using the retrograde horseradish peroxidase (HRP) tracer technique. This study allows comparision of the organization of the hypothalamo-spinal system in hamsters to the corresponding system in other species. Adult golden hamsters were anesthetized with chloral hydrate and their spinal cords exposed by laminectomy. In each animal, at least two 0.3µl injections of 40% HRP were made through a microliter syringe. One injection was made at vertebral level T_1 and another at vertebral level T_2. Injections were unilateral, halfway between the midline and the lateral margin of the spinal cord. The animals were allowed to survive 42-46 hours after the injection, then anesthetized with chloral hydrate and perfused with 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1M phosphate buffer (pH 7.4), followed by 10% sucrose/phosphate buffer at 4°C. Brains and spinal cords were removed, allowed to post-fix and placed overnight in 10% sucrose/phosphate buffer at 4°C. Serial frozen sections (40µ) were cut in the frontal plane and collected in phosphate buffer. The tissue was then processed according to the tetramethylbenzidine technique of Mesulam (J. Histochem. Cytochem. 26: 106-117, 1978). After the reaction was complete, the sections were mounted on chrom alum-gelatin coated slides, and alternate slides were counter-stained with neutral red. The most rostral HRP-labeled cells were observed in the parvocellular division of the paraventricular nucleus (PVN) and in the retrochiasmatic area at the same level. Labeled parvocellular neurons surround the magnocellular division of PVN, which also contains a few large HRP-positive cells. The most dense concentration of HRP-labeled neurons in the PVN is found at the level of the rostral ventromedial hypothalamic nucleus. In addition, a large number of labeled neurons in the lateral parvocellular division of PVN form a bridge between PVN and the lateral hypothalamus (LH). These labeled cells are fusiform in shape with mediolaterally oriented long axes. Scattered HRP-labeled neurons are located in the LH rostral to the formation of the bridge, and at more caudal levels numerous labeled cells are observed in the LH. Labeled cells are also located at the diencephalic-mesencephalic junction. The distribution of labeled neurons observed in the hypothalamus of the hamster after spinal cord injections of HRP corresponds closely to that reported in the rat.

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58.18 AFFERENT CONNECTIONS OF THE DORSAL TEGMENTAL NUCLEUS: A HORSERADDISH PEROXIDASE STUDY. D. Wirtshafter and K. E. Asin Dept. Psychol., Univ. Ill. at Chicago, Chicago, Il 60680 and Kinsmen Lab. of Neurological Research, Univ. British Columbia, Vancouver, BC Canada.

Structures related to the limbic system are known both to project to and receive projections from the paramedian tegmentum of the midbrain, a region which contains structures such as the dorsal and median raphe nuclei and the dorsal and ventral tegmental nuclei of Gudden. Although the raphe nuclei have been the subject of much anatomical investigation, less is known about the connections of Gudden's nuclei. We therefore have examined the connections of the dorsal tegmental nucleus (DTN) using the horseraddish peroxidase (HRP) technique.

Micropipettes (tip diameter: 60-80u) were filled with a 30% solution of HRP (Sigma type VI) and allowed to dry for one hour. The micropipettes were then stereotaxically placed in the DTN and allowed to remain in place for periods of 2-6 min. to allow HRP to diffuse from the tip. Animals were sacrificed following a 24 hr. survival period and brains were processed using tetramethylbenzidine as substrate.

The largest descending projections to the DTN appeared to arise from the interpeduncular nucleus (IPN), the lateral mamillary nucleus and the median raphe. Labeled cells were concentrated along the lateral borders of the IPN although some cells were seen in the central portions. Smaller numbers of labeled cells were seen in the supramamillary region, the ventral portion of the lateral habenula and the periaquaductal grey. Occasional labeled cells were seen in the posterior lateral hypothalamus, the ventral tegmental area and the rostral mesencephalic reticular formation.

The dorsal tegmental nucleus also appears to receive a large ascending projection which originates in the supragenual nucleus. Caudal to this level, occasional labeled cells were seen in the nucleus prepositus and in the central grey.

Some ascending projections of the DTN itself could also be visualized in our material. Both the central and lateral portions of the IPN were densely filled with granules probably representing terminal labeling. Labeled axons could be traced through the mamillary peduncle to the lateral mamillary nucleus which was filled with apparent terminal labeling. Finally, some labeled fibers could be traced into the medial forebrain bundle

58.19 AFFERENTS OF THE VENTRAL PALLIDUM STUDIED WITH A COMBINED IMMUNOHISTOCHEMICAL-ANTEROGRADE DEGENERATION METHOD. L. Zaborszky*[1], G.F. Alheid[1], V.E. Alones*[1], W.H. Oertel [2], D.E. Schmechel*[3], and L. Heimer[1]. 1. Clinical Neurosciences Research Center, University of Virginia School of Medicine, Charlottesville, Virginia 22908. 2. Neurologische Klinik, Technische Universitat Munchen, Munich, Federal Republic of Germany. 3. Division of Neurology, Duke University Medical Center, Durham, North Carolina.

The concept of a ventral striato-pallidal system was introduced by Heimer and Wilson (1975) on the basis of cytoarchitecture, hodology, and histochemistry. We have examined the afferents of the ventral pallidum using a silver technique for anterograde degeneration (Gallyas, Zaborszky, Wolff, 1980) combined with immunochemistry for identical or adjacent brain sections. The PAP or immunofluorescent antibody method was used on frozen sections of formalin perfused rat brains to determine the location of enkephalin (ENK), glutamate decarboxylase (GAD), and substance P (SP) immunoreactivities. ENK-, GAD-, and SP-like immunoreactivities are coextensive in the ventral pallidum with only slight differences in their distribution. The rostro-ventral extension of this immunostaining into the polymorph layer of the olfactory tubercle indicates that the ventral pallidum is more extensive than commonly accepted, in agreement with recent reports (Haber & Nauta, Neurosci. Abs 1981; Switzer et al. 1982).

To examine the origin of these pathways, electrolytic or ibotenic acid-induced lesions were made in the olfactory tubercle, nucleus accumbens, and caudate-putamen. The appearance of degenerating terminals in the ventral pallidum was matched by a substantial decrease in immunostaining, especially for ENK and SP, in the same discrete area. In addition to the pallidal afferents from n. accumbens, and olfactory tubercle, our material suggests that the rostro-ventral caudate-putamen contributes significantly to the innervation of the subcommissural part of the ventral pallidum. The projections from accumbens, caudate and tubercle seem to follow a rough topography. N. accumbens and ventral caudate contribute heavily to the dorsal part of ventral pallidus, while the tubercle projects to the more ventral part. The tubercle maintains its medial to lateral topography in the projection to ventral pallidum.

58.20 WHAT KIND OF CORTEX IS THE HIPPOCAMPUS? V. Braitenberg and A. Schüz*. Max-Planck-Institut für Biologische Kybernetik, Tübingen, W. Germany.

There are good reasons for considering the hippocampus an integral part of the cerebral cortex: the continuity of the axo-dendritic network, the continuation of the intrinsic coordinates of the cortex (the cortical "plane" and the cortical "vertical") into the hippocampus, the predominance of the same (pyramidal) neuron type. Less well known: the predominance of synapses between pyramidal cells mediated by axon collaterals running in all directions of the plane. We illustrate this fundamental isotropy of the hippocampal neuropile in a set of tangential sections. The most important distinguishing features of the hippocampus are: 1) the lack of long range cortico-cortical connections between pyramidal cells, 2) the alignment of pyramidal cells in one narrow layer, eliminating any top to bottom bias and making the narrow range connectivity truly 2-dimensional, 3) the presence of the well known unidirectional, probably re-entrant pathway: perforant path, mossy fibers, Shaffer collaterals. Our quantitative estimates (referring to the mouse hippocampus) lead to the following conclusions. The unidirectional pathway commands only a small fraction (about 1%) of all synapses. Each dentate granular cell contacts 50 hippocampal pyramidal cells. Each pyramidal cell is contacted by up to 200 different granular cells. An individual pyramidal cell is contacted by about 18,000 synapses (about three times more than in the rest of the cortex). Thus the unidirectional pathway introduces but a slight anisotropy into the hippocampal neuropile. From the standpoint of cortical architecture, we notice that the hippocampus proper is a continuation of the lower cortical layers, the upper layers stopping at the hippocampal "cliff" in the presubiculum. Since generally much of the input to the lower layers stems from the upper layers, the presence of the perforant path which originates in the upper entorhinal layers is in keeping with this. Ammon's horn appears as a protruding tongue of the lower entorhinal layers, carrying its input fibers with it.

59.1 KETAMINE AND N-ALLYLNORMETAZOCINE INTERACT SIMILARLY WITH MULTIPLE OPIATE BINDING SITES IN SPINAL CORD TISSUE. D.J. Smith & R.L. Bouchal*, Depts of Anesthesiology & Pharmacology, West Virginia University Medical Center, Morgantown, WV 26506.

The dysphoric, intravenous anesthetic agent, ketamine, interacts with opiate receptors as an agonist (Smith et al., Life Sci. 26:789, 1980). In the present study, the drug's interaction with opiate receptor sub-types was analyzed and compared to other ligands presumed to be specific for these multiple receptors.

Assays were established with radioligands for the putative μ (^{3}H-dihydromorphine), K (^{3}H-ethylketocyclazocine), δ (^{3}H-leucine enkephalin) and σ (^{3}H-n-allylnormetazocine) opiate receptors. Membranes from rat spinal cord were incubated (18 mg tissue in 0.05 M Tris-HCl buffer, pH 7.7, 25°C) with 1nM of each radioligand. The incubation was terminated by collecting the membranes on Whatman GF/B filters. Radioactivity was determined using Dimilume scintillation medium. Specific opiate receptor binding (total binding-binding in the presence of excess unlabeled homologous ligand) was determined and used to calculate IC 50 values (concentration of displacing ligand reducing specific binding by 50%) and Hill coefficients for displacement curves.

Ketamine displaced each of the radioligands studied but exhibited low potency and IC 50 values that fell within a narrow range (μM of ketamine for the displacement of ^{3}H-dihydromorphine, 27±7.6; ^{3}H-ethylketocyclazocine, 85±26; ^{3}H-n-allylnormetazocine, 66±10 and ^{3}H-leucine enkephalin, 100±9.0). N-allylnormetazocine was qualitatively similar to ketamine in each binding assay, although the former drug was more potent (IC 50 values of about 10 nM). Both drugs generated monophasic displacement curves, regardless of the radioligand being studied, with Hill coefficients near unity. Coefficients greater than 0.8 suggest that a radioligand is being displaced from a single population of binding sites or that a displacing drug has a similar affinity at multiple radioligand binding sites. The latter appears to be most likely for ketamine and n-allylnormetazocine, since other displacing drugs (morphine, ethylketocyclazocine, naloxone and leucine enkephalin) distinguished multiple binding sites in at least two of the assays (^{3}H-leucine enkephalin and ^{3}H-n-allylnormetazocine). With these other drugs it was observed that displacement was multiphasic or generated Hill coefficients less than 0.8.

Ketamine, therefore, interacts with several sub-types of opiate receptors but without a marked preference. Its interactions appear qualitatively similar to those of the hallucinogenic σ receptor ligand n-allylnormetazocine. Thus, ketamine may induce dysphoric reactions by behaving as a sigma ligand to produce an opiate-linked psychosis.

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59.2 LOCALIZATION OF A POPULATION OF OPIATE RECEPTORS ON STRIATAL SEROTONINERGIC NERVE TERMINALS. M.Parenti, L.Dellavedova+, L.Vicentini+ and A.Groppetti+. Inst. of Pharmacology, Univ. of Milan, Sch. of Medicine, Milan, Italy.

Several investigators have shown that morphine and opioid peptides affect the activity of brain serotoninergic (5-HT) neurons.

Now we report evidence that in striatum this interaction may be locally mediated by opiate receptors present in this brain area.

1) Shortly after mechanical interruption of the raphe-striatal 5-HT fibers, at a time when most of the metabolic processes are still operative in the lesioned neurons, morphine does not lose its ability to increase striatal content of 5-hydroxyindoleacetic acid.

2) After degeneration of 5-HT neurons by 5,6-dihydroxytryptamine (5,6-DHT) administration or transection between striatum and raphe nuclei, the number of binding sites for (^{3}H)D-Ala^2-met^5-enkephalinamide (^{3}H-D-Ala^2) is significantly reduced (Table).

This latter effect seems to be related neither to the opiate receptors that have been indicated to be present on dopaminergic (DA) terminals (Pollard et al., Nature, 268: 745, 1977), whose activity does not result to be significantly affected by the lesions (Table), nor to a simple decrease in 5-HT neuronal tone since after p-chlorophenylalanine (100 mg/kg i.p. x 4 days) the number of binding sites for ^{3}H-D-Ala^2 in striatum is not diminished.

The data in table may therefore indicate that a population of opiate receptors is located on striatal 5-HT terminals where possibly modulates the presynaptic activity of these 5-HT neurons.

In this context we have found that morphine pretreatment facilitates while naloxone antagonizes the releasing effect of fenfluramine on striatal 5-HT.

Table - ^{3}H-D-Ala^2 binding, 5-HT and DA levels in rat striatum

	Bmax pmole/mg prot	5-HT μg/g tissue	DA μg/g tissue
Control	0.60 ± 0.04	0.79 ± 0.03	10.40 ± 0.60
5,6-DHT	0.42 ± 0.04+	0.46 ± 0.03++	11.20 ± 0.80
Transected	0.46 ± 0.03+	0.29 ± 0.02++	11.02 ± 0.87

+ $p < 0.05$ ++ $p < 0.01$

^{3}H-D-Ala^2 binding was performed on striatal crude particulate pellet. K_D was not significantly different in the 3 groups (K_D = 3.4 ± 0.2 nM for controls)

5,6-DHT: 150 μg/rat i.v.t.; 10 days before sacrifice.

Transection: knife 2.5 mm wide (A = 1.0; P = -2.0; L = 0.0 according to König-Klippel rat brain atlas); 2 weeks before sacrifice.

59.3 IN VIVO/IN VITRO γ-ENDORPHIN INTERACTION WITH DOPAMINERGIC LIGANDS IN RAT BRAIN. E.E. Codd*, H. Scholtens*, G. Wolterink*, J. Verhoef*, J.M. van Ree* and A. Witter* (SPON: Tj.B. van Wimersma Greidanus). Rudolf Magnus Inst. Pharmacol., Vondellaan 6, 3521 GD Utrecht, The Netherlands.

While the β-endorphin fragments 2-17 (DTγE) and 6-17 (DEγE), have neuroleptic-like effects in laboratory animals and exhibit antipsychotic activity in schizophrenic patients, the mode of action underlying these effects is not yet established. In vitro studies have been unsuccessful in finding an interaction between DTγE (Eur. J. Pharmacol. 52:411, 1978; Life Sci. 24: 1645, 1979a) or DEγE (unpublished results) and brain dopaminergic/neuroleptic binding sites. However, DTγE was reported to interact with in vivo ^{3}H-spiperone binding (Eur. J. Pharmacol. 60: 359, 1979b). The apparently conflicting results from in vitro and in vivo experiments stimulated attempts to replicate the in vivo experiments and to extend these studies to conditions currently in use for assessment of γ-type endorphin behavioral activity.

The first series of experiments used the model of Pedigo et al. (1979b) in which striatal displacement of intravenously administered ^{3}H-spiperone by subcutaneously administered haloperidol or DTγE was monitored. Measures were made not only of total radioactivity present in the striatum but also of radioactivity bound to a particulate fraction and of radioactivity in the plasma. Haloperidol treatment significantly decreased the total and bound ^{3}H-spiperone in the striatum and elevated the amount of ^{3}H-spiperone in the plasma. DTγE treated animals tended to have lower amounts of striatal and particulate bound ^{3}H-spiperone and slightly elevated plasma ^{3}H-spiperone levels, but these effects were not statistically significant.

In the second series of experiments, drugs were administered directly into the nucleus accumbens. DEγE rather than DTγE was used and ^{3}H-apomorphine was used as the ligand in this series. DEγE administration resulted in marked decreases in the bound ^{3}H-apomorphine although these effects did not reach statistical significance because of large variations between animals. Plasma ^{3}H-apomorphine was significantly elevated after DEγE administration.

It is concluded that the interaction between γ-type endorphins and dopaminergic binding may be either indirect or limited to a subset of these sites.

59.4 COMPARISON OF ^{3}H-DIPRENORPHINE BINDING IN RAT BRAIN AND SPINAL CORD. K. J. Mack, A. Killian*, and J. A. Weyhenmeyer. College of Medicine and Neural and Behavioral Biology Program, University of Illinois, Urbana.

^{3}H-Diprenorphine, an opiate antagonist which binds to mu, delta and kappa binding sites with similar affinity, was used to investigate opiate receptor binding in rat brain and spinal cord. Membrane preparations from male adult albino Sprague-Dawley rats were incubated with levels of ^{3}H-diprenorphine from 0.1 nM to 5.0 nM for 60 minutes, and then washed and filtered on Whatman GF/C filters. Scatchard plots revealed an affinity constant, K_D, for rat brain (minus cerebellum and brainstem) of 0.295 ± 0.020 nM and a maximum binding, B_{max}, of 639 ± 71 fm/mg. Rat spinal cord had a lower affinity of 0.502 ± 0.037 nM and a lower B_{max} of 221 ± 23 fm/mg.

Addition of 10 uM concentrations of the specific mu agonist morphiceptin has previously been used to block mu receptors in brain tissue (Chang et al, Proc. Natl. Acad. Sci. 78:4141, 1981). In rat CNS membranes, we found that 10 uM morphiceptin blocked 56.9 ± 4.0 % of ^{3}H-diprenorphine binding to brain and blocked 71.2 ± 4.7 % of spinal cord binding. Further addition of 0.1 uM D-ala^2-D-leu^5-Enkephalin to block delta receptor binding, reduced total binding by an additional 26.1 ± 2.9 % in brain and 6.9 ± 3.5 % in spinal cord. The remaining binding of 17.0 ± 2.0 % in brain and 21.9 ± 3.8 % in spinal cord is probably not mu, not delta binding and may represent benzomorphan or kappa binding sites.

These results suggest differences in opiate binding between rat brain and spinal cord. ^{3}H-Diprenorphine binds to higher affinity sites in brain vs. cord ($p<0.01$) as determined by scatchard plots. However, since the scatchard analysis may not always seperate two or three binding sites with similar affinity, part of this observed difference may be explained by the tentative differences in opiate receptor subtype populations.

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59.5 OPIATE RECEPTOR SUPERSENSITIVITY PRODUCED BY CHRONIC NALTREXONE: ELECTROPHYSIOLOGIC EVIDENCE IN LOCUS COERULEUS. M.T. Bardo, R.K. Bhatnagar and G.F. Gebhart. Dept. of Pharmacology, University of Iowa, Iowa City, IA 52242.

Rats were implanted subcutaneously with either naltrexone (10 mg) or placebo pellets for 28 consecutive days. The pellets were then removed and, one day later, each rat was assessed either for specific binding of opiates in various CNS regions or for opiate-induced inhibition of neuronal firing in the brainstem locus coeruleus (LC). For assessment of opiate binding, animals were decapitated, the brains and spinal cords removed, and brains regionally dissected into medulla-pons, midbrain, hypothalamus, striatum and cortex. Tissue was homogenized in Tris HCl buffer, pH 7.4, and incubated at 0°C for 180 min. with 1 nM ^{3}H-naloxone in the presence or absence of 100 nM levallorphan. Each sample was then washed over GF/B glass fiber filters and the radioactivity was determined by liquid scintillation spectrometry. For extracellular recording of LC neurons, animals were anesthetized with chloral hydrate and a single-barrel glass electrode (1-2 μ tip) filled with fast green dye was placed stereotaxically in the LC (1.2 mm L; 1.4 mm P; 6.0 mm below dura). After obtaining a baseline spontaneous firing rate for a single unit, morphine was administered i.v. in incremental doses (0.1 to 1.2 mg/kg), followed by incremental doses of i.v. naloxone (0.01 to 0.10 mg/kg). Drug-induced changes in firing rate were expressed as a difference from baseline spontaneous activity.

Naltrexone pellet implantation produced a significant increase in specific binding of ^{3}H-naloxone in spinal cord (39%), medulla-pons (30%), hypothalamus (35%) and striatum (51%). Nonsignificant increases in ligand binding were also evident in midbrain (30%) and cortex (25%) following naltrexone pellet implantation. Concomitant with this increase in opiate binding, naltrexone pellet implantation produced a significant decrease in the baseline firing rate of LC neurons. The mean spontaneous firing rate of LC neurons was 1.32 spikes/sec in naltrexone treated rats (n = 12 cells) and 2.36 spikes/sec in placebo treated rats (n = 8 cells). Morphine significantly inhibited LC neuronal activity in a dose-dependent manner in both naltrexone and placebo treated rats. The magnitude of inhibition produced by each dose of morphine was significantly greater in naltrexone treated rats than in placebo treated rats. Naloxone reversed the inhibitory effect of morphine in both naltrexone and placebo treated rats. These results demonstrate that chronic blockade of brain opiate receptors with naltrexone produces an increase in the number of receptors and a concomitant supersensitivity of LC neuronal activity to the inhibitory effects of morphine.

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59.6 UM 1071 BINDING IN RAT AND GUINEA PIG BRAIN: THE EFFECTS OF ALKALOIDS AND ENDOGENOUS OPIATE LIGANDS. E. Young*, J. Woods, and H. Akil. (SPON: J. WOODS). Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109

The existence of subtypes of opiate receptors was postulated a number of years ago. The mu and delta receptor subtypes have been easy to demonstrate with binding procedures; it has been more difficult to demonstrate kappa receptors in rat brain, although experiments using guinea pig (GP) brain have generally been more successful. We have used ^{3}H UM 1071, the active stereoisomer of the benzomorphan MR 2034, in both GP and rat brain simultaneously. This compound has been characterized behaviorally as a kappa agonist. It labels a site which is more concentrated in GP brain (Bmax rat = 418 pM; Bmax GP = 829 pM). Other putative kappa ligands compete well with this compound. The regional distribution of UM 1071 sites is similar to that of morphine binding with a relatively uniform density across brain regions except striatum which shows more UM 1071 sites than other areas. However, this ligand does not appear to be labelling a mu site, since typical mu compounds do poorly in competing against it.

The potencies of several alkaloids and opioid peptides were compared in GP and rat brain. When competing against ^{3}H morphine there were no significant interspecies shifts. However, competition against ^{3}H UM 1071 showed different patterns in the two species. In general, mu and delta ligands lost potency and kappa ligands gained relative potency in GP brain. Thus, DADL, Met-Enk-Arg-Gly-Leu, and β-endorphin, all delta-like potencies, were 3 to 6 fold less potent in displacing UM 1071 in GP. Morphine, the prototypical mu, was 3 times less potent. In contrast, MR 2266, the etorphine analogue UM 928, as well as EKC, all presumed kappa, either remain equipotent or gain in potency when competing against ^{3}H UM 1071 in GP as compared to rat. Interestingly, dynorphin-(1-13), dynorphin-(1-17) and α-neo-endorphin, all gain potency in the GP, suggesting a kappa-like property.

Thus it appears that ^{3}H UM 1071 binds to kappa sites in both rat and GP brain; however, since there are more kappa sites in GP brain, UM 1071 labels these sites more selectively, rendering it more difficult for mu and delta ligands to displace it in that tissue. Thus, the ratio of IC_{50} GP: IC_{50} rat brain vs. ^{3}H UM 1071 appears to be a useful discriminator for kappa compounds.

59.7 ALTERATION OF MEMBRANE SULFHYDRYL GROUPS INVOLVED IN OPIATE RECEPTOR COMPLEXES IN RAT BRAIN. S. R. CHILDERS AND J. L. JACKSON*. Dept. Pharmacology, Univ. of Florida College of Med., Gainesville, FL 32610.

Previous experiments using selective ligands to protect vital SH groups in membranes from alkylation by N-ethylmaleimide (NEM) have shown that opiate receptors consist of several separate, but interacting, sites: 1) receptor binding site; 2) GTP regulatory site; 3) sodium regulatory site. Since NEM reacts with the S(-) rather than the SH form of the sulfhydryl group, we attempted to determine the apparent pK of each site by incubating NEM with membranes at different pH values. The receptor site was measured with ^{3}H-D-Ala met enkephalinamide (D-Ala enk) binding, while the GTP and sodium sites were measured by inhibition of binding by 50 μM GTP and 100 mM NaCl, respectively. NEM (250 μM) was incubated with rat brain membranes at various pH values at 25° for 15 min, the reaction terminated by 2 mM dithiothreitol, and membranes washed twice with pH 7.7 buffer before assay. NEM caused a 60% reduction of ^{3}H-D-Ala enk binding at pH 7.0; increasing the pH to 8.5 had no effect on NEM-induced decrease in binding, while decreasing pH below 6.0 caused a gradual decrease in the effectiveness of NEM. At pH 4.2, NEM inhibited binding by less than 10%, with an apparent pK of approx. 5.5. Pre-incubation of membranes between pH 4.2 and 8.5 without NEM had no significant effect on binding, but low pH pre-treatment (pH 4.2-6.0) increased both sodium and GTP effects on binding by approx. 25%. NEM reduced the GTP inhibition of binding by 50% at pH 7.0, and, like the binding site, NEM was gradually less effective in reacting with the GTP site at lower pH values, with an apparent pK, like the receptor, of approx. 5.5. NEM increased the sodium effect at pH 6.0-8.5, but was totally ineffective at pH below 5.8. Interestingly, assay of total SH groups in membranes with Ellman's reagent demonstrated that NEM reacts with only 15% of total membrane SH groups at pH 4.2-6.0, with no effect of pH on NEM reactivity with total SH groups. These results suggest that SH groups in the opiate receptor complex, which react with NEM at neutral pH values, are inaccessible at low pH values, a property not observed with most membrane SH groups which can react with NEM.

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59.8 LIGHT AND OXYGEN INDUCED ARTIFACTS IN ETORPHINE BINDING. William F. Herblin. E. I. du Pont de Nemours & Co., Glenolden, PA

Etorphine is a useful ligand for studies of opiate receptors because it binds with high affinity to several of the reported sub-types. Naloxone is the prototype opiate antagonist and is therefore commonly used as a standard in opiate binding studies. During our evaluation of the binding of etorphine under a variety of conditions, we observed an apparent potentiation of etorphine binding by naloxone. Low concentrations of naloxone (10^{-8}-10^{-7}M) added to the incubation mixture reduced the specific binding of etorphine by 3-40% but when the concentration was increased to 10^{-6}M or above, the inhibition disappeared and the counts retained on the filter increased by up to 60%.

Investigation of this phenomenon indicated that it is due to an unidentified chemical process which occurs even in the absence of tissue and is specific for naloxone and naltrexone of the several opiates tested. The exclusion of light and oxygen during the incubation can prevent the reaction. The mechanism is not known but could be a direct interaction of naloxone and etorphine or a naloxone potentiation of etorphine degradation.

59.9 IN VITRO RECEPTOR SELECTIVITY PROFILE AND THE IN VIVO CONSTIPATIVE EFFECTS OF OPIATES. A. Pierson*, W. Cumiskey*, D. Wescoe*, H. Lawyer*, E. Baizman*, W. Michne* and M. Feigenson* (SPON: J. Saelens). Sterling-Winthrop Res. Inst., Rensselaer, NY 12144.

It has been suggested that the antidiarrheal effect of opiates is mediated via peripheral receptors of the delta type located in ileal muscle and the ileocecal sphincter (Cardwell et al, Gastroenterology 80:1120, 1981). Selected benzazocine and peptide opiates were studied to determine if those exhibiting delta selectivity (DS) in vitro are more effective inhibitors of gastrointestinal propulsion (GIP) in vivo than those relatively more mu selective. Receptor selectivity ratios+ were obtained from field stimulated guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations for the following compounds: D-Ala^2-D-Leu^5-enkephalin (DADLE), Leu^5-enkephalin (LE), LY 127623 (LY), FK 33-824 (FK), morphine (M), (2α,6α, 11S*)-(±)-1-(1, 2, 3, 4, 5, 6-hexahydro-8-hydroxy-3, 6, 11-trimethyl-2, 6-methano-3-benzazocin-11-yl)-3-phenyl-3-propanone (I), (2α, 6α, 11S*)-(±)-1, 2, 3, 4, 5, 6-hexahydro-3, 6, 11-trimethyl-11 octyl-2,6-methano-3-benzazocin-8-ol (II), (2S, 6R, 11R)-6-ethyl-3, 4, 5, 6-tetrahydro-8-hydroxy-11-methyl-2, 6-methano-3-benzazocin-1-(2H)-one (III), and (2α, 6α, 11S*)-(±)-4-(1, 2, 3, 4, 5, 6-hexahydro-8-hydroxy-3, 6, 11-trimethyl-2, 6-methano-3-benzazocin-11-yl)-2-butanone (IV). Equieffective antinociceptive doses** of I, II, III, IV, LY, FK, and M were tested for effects on intestinal advancement of a charcoal meal (mouse). In descending order of DS: DADLE > LE > LY > I >II > FK > III > M > IV. In descending order of inhibitory effect on GIP: FK > LY > I > II, M, IV > III. Generally, rank ordering of opiates in terms of DS was similar to rank ordering in terms of GIP inhibition. However, LY was about 6x more delta selective than FK even though they affected GIP similarly. Further, III and M had similar DS ratios yet III was markedly less inhibitory on GIP. These results do not clearly support or refute the hypothesized role of intestinal delta receptors in the constipative effect of opiates.

Compound	In Vitro Selectivity Ratio+	In Vivo % ± S.E. Inhibition GIP**
DADLE	4	-
LE	11	-
LY	59	71 ± 2.1
I	100	59 ± 2.3
II	245	51 ± 2.0
FK	342	76 ± 1.5
III	473	18 ± 4.2
M	500	50 ± 3.6
IV	764	49 ± 5.3

+Quotient of potency relative to M in GPI/potency relative to DADLE in MVD.
**Dose = ED_{90} from acetylcholine-induced writhing assay (mouse).

59.10 RADIOAUTOGRAPHIC VISUALIZATION OF SPECIFICALLY LABELED OPIATE RECEPTOR SITES IN FIXED BRAIN SECTIONS. Edith Hamel and Alain Beaudet, Lab. of Neuroanatomy, Montreal Neurological Inst., McGill University, Montreal, Quebec H3A 2B4.

The utilization of radiolabeled met-enkephalin analogue FK 33-824 or its N-methyl-tyrosine derivative FW-569 (Sandoz) for visualizing opiate receptor sites by high resolution radioautography was investigated in rat brain tissue sections.

Optimal conditions for specific binding of ^{125}I-FK and ^{3}H-FK were first determined biochemically on unfixed, frozen sections. At room temperature, both ligands showed optimal binding at 30 min and the affinity for the ligands (K_D) was close to 1nM. The binding was unaffected by prior fixation of the brain by intra-aortic perfusion of 0.1% glutaraldehyde/0.1% paraformaldehyde. Examination of CNS binding sites by film radioautography revealed that ^{3}H-FK, ^{125}I-FK and ^{125}I-FW all showed identical topographical distributions. In agreement with previous reports, high densities of labeled opiate receptor sites were detected in the thalamus, in several brain stem nuclei (particularly in locus coeruleus) and in circumscribed areas of the striatum.

The effects of different post-fixation procedures on ligand binding and tissue integrity were subsequently analysed biochemically and/or by radioautography. Tissue sections incubated with ^{3}H-FK or ^{125}I-FK retained more than 70% of specifically bound FK after fixation by immersion in 6% glutaraldehyde. In addition, close to 75% of the remaining bound FK was retained after delipidation in alcohols and xylene. Both film and light microscope radioautography showed no change in receptor site distribution following either of these procedures. Similarly, a high proportion of specifically bound ^{125}I-FK was held without displacement in sections fixed with glutaraldehyde, postfixed in 2% osmium tetroxyde and dehydrated in ethanols. In contrast, the methylated derivative FW, though also partially retained after post-fixation in glutaraldehyde, was completely washed out in the course of delipidation.

These data indicate that 1) ^{3}H-FK, ^{125}I-FK and ^{125}I-FW exhibit comparable binding characteristics to rat brain opiate receptors 2) the use of ^{125}I or ^{3}H-FK makes it possible to analyse, in conditions ensuring relatively good morphological preservation, the topographic distribution of CNS opiate receptor sites by standard "wet" radioautographic techniques. 3) utilization of the same fixation procedures after incubation of fresh tissue sections under physiological conditions should allow localization of these opiate receptor sites at electron microscopic level. (Supported by a Fellowship (E.H.) and grant MA 7366 from the Medical Research Council of Canada.)

59.11 LIGHT MICROSCOPIC AUTORADIOGRAPHY OF MU AND DELTA OPIATE BINDING SITES IN MOUSE CNS. R.R. Goodman, M.J. Kuhar and A.S. Moskowitz. Dept. of Neuroscience and Pharmacology and Experimental Therapeutics, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205 and Brain Research Institute and Psychology Dept., UCLA, Los Angeles, CA 90024.

The existence of multiple opiate binding sites is supported by evidence from binding studies and bioassays (Kosterlitz et al, Br. J. Pharmacol., 179: 333 - 342, 1980). The mu site has a higher affinity for opiate alkaloids, whereas the delta site has a higher affinity for enkephalins. Using the in vitro light microscopic autoradiography technique of Young and Kuhar (Brain Res., 179: 255 - 270, 1979) we have compared the distribution of mu (Labeled by 3H dihydromorphine) and delta (labeled by 3H d-ala^2-d-leu^5-enkephalin) sites in the CNS of two mouse strains (C57BL/6BY and CXBK, Jackson Labs). The CXBK strain has a lower number of whole brain opiate binding sites and shows less analgesia in response to systemically administered morphine than the C57 strain (Baran et al, Life Sci., 17: 633 - 640, 1975).

The results from the C57 strain correlate well with results obtained in the rat (Goodman et al, Proc. Natl. Acad. Sci. U.S.A. 77: 6239 - 6243, 1980). Mu sites are predominant in laminae I, IV and VI of the neocortex, in patches in the caudate/putamen, in the medial thalamus, hypothalamus, superior colliculus, periaqueductal gray matter and laminae I and II of the spinal cord. Delta sites are more diffusely distributed with high concentrations found in laminae I - VI of the neocortex, rostral levels of the caudate/putamen and the olfactory tubercle. Preliminary analysis indicates that the CXBK strain has a noticeably lower level of mu sites in the caudate/putamen, substantia nigra, periaqueductal gray matter and laminae I and II of the spinal cord. Supported by Mental Health Training Grant #MH 15345, NIH grant #NS 07628 and UPHS grants DA-00266 and MH-00053.

59.12 INTERACTIONS BETWEEN MULTIPLE OPIOID BINDING SITES: USE OF β-FUNALTREXAMINE (β-FNA), A HIGHLY SELECTIVE NON-EQUILIBRIUM μ ANTAGONIST. A.E. Takemori, Susan J. Ward, P.S. Portoghese. Departments of Pharmacology and Medicinal Chemistry, University of Minnesota, Minneapolis, Mn 55455.

Selective binding sites for μ, κ and δ opioid ligands have been demonstrated in brain membranes, and it has recently been suggested that interaction at δ binding sites may allosterically inhibit the binding of ligands to μ binding sites (Rothman and Westfall, Eur. J. Pharmacol., 72, 365-8, 1981). In the present study, mouse brain membranes were incubated in the presence and the absence of 1 μM β-FNA and then washed 3 times to remove reversibly bound β-FNA. In competition experiments, β-FNA pretreatment reduced the ability of low concentrations (0.3 - 10 nM) of morphine to inhibit the binding of ^{3}H-naltrexone (1 nM) and ^{3}H-D-Ala^2-D-Leu^5-enkephalin (^{3}H-DADLE)(1 nM). The Hill plot for the interaction of morphine and ^{3}H-DADLE was reduced from a biphasic plot to a single line, and the lower half of the Hill plot for the interaction of morphine and ^{3}H-naltrexone was shifted rightward. The ability of both low and high concentrations (0.3 nM - 10 μM) of morphine to inhibit the binding of ^{3}H-ethylketazocine (^{3}H-EK)(1 nM) were reduced in membranes pretreated with β-FNA, and the Hill plot for the interaction was shifted rightward. Pretreatment with β-FNA also decreased the ability of high (0.1-10 μM) concentrations of EK to inhibit the binding of ^{3}H-EK and ^{3}H-DADLE, but not that of ^{3}H-naltrexone. The slopes of the upper portion of the single line Hill plots for the interactions of EK with ^{3}H-EK and ^{3}H-DADLE were diminished by β-FNA pretreatment. A similar diminution of the upper half of the Hill plot was seen for the interaction of DADLE with ^{3}H-DADLE since the inhibitory effects of high (0.1 - 10 μM concentrations of DADLE upon ^{3}H-DADLE binding were decreased by β-FNA pretreatment. Pretreatment of membranes with β-FNA had no effect upon the inhibitory actions of DADLE on the binding of ^{3}H-naltrexone or ^{3}H-EK. It is concluded that occupation of μ binding sites can markedly affect the interactions of various opioid ligands with their binding sites, and that the high affinity binding sites of all the radiolabelled ligands studied are sensitive to μ binding site occupation.

This study was supported by U.S. Public Health Grants DA 00289 and DA 01533 from the National Institute on Drug Abuse.

59.13 EVIDENCE FOR THE PRESYNAPTIC LOCALIZATION OF DELTA OPIATE RECEPTORS ON THE STRIATAL EFFERENTS. B. Abou-Khalil*, A.B. Young and J.B. Penney, Dept. of Neurology, University of Michigan, Ann Arbor 48109

The differential localization of mu and delta opiate receptors have been studied biochemically and autoradiographically. In the striatum, mu receptors are concentrated in clusters and delta receptors are more evenly distributed. Studies using 6-hydroxydopamine lesions of substantia nigra suggest that the clusters of mu receptors in the striatum exist on dopaminergic terminals (Murrin et al., Life Sci 27:1175, 1980). The localization of delta receptors in striatum has not been clearly defined. Using quantitative receptor autoradiography, we have studied mu and delta receptors in striatum, and areas to which striatal cells project (globus pallidus (GP) and substantia nigra pars reticulata (SN_r)) of rat after unilateral kainic acid lesions of striatum.

Six Sprague-Dawley rats (125-175 g) were lesioned unilaterally in the striatum using microelectrophoresis of 1 nmole of kainic acid at the level of the anterior commissure. The rats were decapitated 7-11 days after the operation and their brains were frozen in dry ice. Twenty micron frozen sections of brain were mounted on subbed slides and the slides were washed in Tris HCl buffer pH 7.4 at 4° C and then dried. Subsequently, sections were incubated either with [^{3}H] naloxone (1-4 nM) in 50 mM Tris HCl and 100 mM NaCl at 4° C for 1 hour, or with [^{3}H] D-ala-D-leu-enkephalin (DADL) (2-8 nM) in 50 mM Tris HCl, 30 mM NaCl, 3 mM manganese acetate and 2 μM GTP at 20° for 30 min. The slides were then given six 20 second washes with Tris HCl, pH 7.4 at 4° C, dried, mounted in an x-ray cassette with standards and tritium-sensitive film. The film was exposed for 3 weeks at 4° C. After development, mu and delta receptors were determined densitometrically (Penney et al., Science 214:1036, 1981).

Delta and mu opiate receptors were measured respectively using [^{3}H]DADL and [^{3}H]naloxone at the stated incubation conditions. In the lesioned striatum, delta receptors were reduced to 38 $\pm$ 3% and mu receptors to 83 $\pm$ 4% of the control side. In GP and SN_r, delta receptors were reduced to 74 $\pm$ 7% and 65 $\pm$ 3% of control and mu receptors were unchanged. The reduction in delta receptors as compared to the mu receptors was significant at $p<.01$ in all regions. The data suggest that delta receptors exist on presynaptic terminals of striatal neurons and that delta receptors can be altered independent of changes in mu receptors. The presynaptic location of delta receptors could potentially be utilized as an in vivo measure of striatal neurons.

This work was supported by a Neuroscience Development Award from the McKnight Foundation and USPHS grants NS00464-03 and NS00420-03.

59.14 TRITIATED β-ENDORPHIN BINDING IN RAT BRAIN: DIFFERENTIAL DISTRIBUTION OF BINDING SITES ACROSS BRAIN REGIONS. William Hewlett*, Huda Akil[1], Walter Carlini*, Jack Barchas, and C. H. Li[2]. (SPON: R. Britt). Nancy Pritzker Laboratory of Behavioral Neurochemistry, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, California 94305. [1]Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan 48109, and [2]Hormone Research Laboratory, University of California, San Francisco, California 94143.

Several sub-classes of opiate receptors have been reported to exist in the CNS and a variety of other mammalian tissues. In order to characterize the opiate binding site sub-classes, the differential distribution of these opiate binding sites has been studied in homogenates prepared from five regions of rat brain. The binding of tritiated β-endorphin (^{3}H-β-END) was compared to the binding of ^{3}H-morphine, ^{3}H-ethylketocyclazocine (^{3}H-EKC), and ^{3}H-[d-ala^2,d-leu^5]-enkephalin (^{3}H-DADLE). Striatum and frontal cortex contain the highest densities of ^{3}H-β-END labeled sites followed by hippocampus, hypothalamus, and midbrain. However, while the ratio of ^{3}H-β-END to ^{3}H-DADLE binding varies by almost four-fold across brain regions, the ratio of ^{3}H-β-END to ^{3}H-EKC binding remains almost constant across these five brain regions. The ability of DADLE, dynorphin(1-13), β-endorphin, morphine, EKC, and N-allyl-normetazocine (SKF) to displace ^{3}H-β-END binding was also studied in the same five regions of rat brain. Dynorphin(1-13) and β-endorphin are the most potent ligands against ^{3}H-β-END across brain regions, followed by SKF. Morphine shows a three-fold variation in potency across regions being most potent in hippocampus and least potent against ^{3}H-β-END in striatum. DADLE shows a two-fold variation in potency across regions, being most potent in striatum and least potent in hypothalamus and midbrain where it is weaker than any other of the unlabeled ligands. The selectivity of the different unlabeled opiates for different binding sites is assessed by examining the ratio of potencies of the unlabeled opiates against different radiolabeled opiates and is discussed in the context of current models for multiple opiate receptors.

60.1 EFFECTS OF ACUTE AND CHRONIC ANTIEPILEPTIC AGENTS ON HYPOTHALAMIC BETA-ENDORPHIN AND OTHER NEUROPEPTIDES. A. Martini, A.M. Di Giulio, A.E. Panerai. Dept. Pharmacology, School of Medicine, U. of Milano Italy.

Since it was suggested that neuropeptides and mainly endogenous opiates might be involved in epilepsies, we evaluated the effects of antiepileptic agents on hypothalamic concentrations of Beta-endorphin, Somatostatin, Substance P and met-enkephalin. Male rats were treated acutely or twice dayly for fifteen days with either Phenobarbital (70 mg/kg acute, 30 mg/kg chronic); Diphenylhydantoin (120 mg/kg acute, 50 mg/kg chronic); Ethosuximide (400 mg/kg acute, 150 mg/kg chronic) or Sodium Valproate (400 mg/kg acute, 150 mg/kg chronic). Moreover, the same peptides were evaluated in the hypothalamus of rats treated with either Aminooxyacetic acid (25 mg/kg acute, 5 mg/kg chronic), Ethanolamine-O-sulphate (2 mg/kg acute), or THIP (20 mg/kg acute). All drugs were administered intraperitoneally with the exception of Ethanolamine-O-sulphate that was administered intraventricularly. Beta-endorphin concentrations were also evaluated in the pituitary after all treatments. Rats were killed by microwave irradiation and the peptides evaluated by radioimmunoassay; representative samples were evaluated also after HPLC separation in order to check for assay specificity. All agents used, with the exception of THIP induced a significant decrease of hypothalamic Beta-endorphin, while Somatostatin, met-enkephalin and Substance P were not affected. Pituitary concentrations of Beta-endorphin increased after Phenobarbital, Diphenylhydantoin and Ethanolamine-O-sulfate, decreased after Ethosuximide and did not change after either Aminooxyacetic acid, Sodium Valproate or THIP. Beta-endorphin concentrations still significantly decreased in the hypothalamus after chronic treatment with Sodium Valproate or Aminooxyacetic acid, while did not change after the other treatments. Somatostatin, met-enkephalin and Substance P did never change after any treatment. The pituitary concentrations of Beta-endorphin still increased after diphenylhydantoin while were not affected by the other treatments. It is interesting observe that the only agents that induce an effect on hypothalamic Beta-endorphin after chronic treatment have similar effects on GABA concentrations since both inhibit the GABA transaminase and Sodium Valproate has also been suggested to increase the activity of glutamic acid decarboxylase i.e. to increase GABA synthesis.

In conclusion, 1) antiepileptic agents seem to specifically affect hypothalamic Beta-endorphin concentrations when administered acutely; 2) an increase of GABA might be important for the effect of Sodium Valproate on Beta-endorphin concentrations during chronic treatment.

60.2 ACUTE AND CHRONIC STRESS: EFFECT ON REGULATION OF THE BETA-ENDORPHIN/ACTH SYSTEM IN PITUITARY AND BRAIN. H. Shiomi, H. Akil, J. Matthews, and S.J. Watson (SPON: G.C. Quarton). Mental Health Res. Inst., University of Michigan, Ann Arbor, MI 48109.

Beta-endorphin and its biosynthetic family (the pro-opiomelanocortin or POMC system) are thought to be released by stress from the anterior lobe of the pituitary and by the hypothalamus. Acute footshock stress which has certain characteristics is indeed known to produce analgesia which is naloxone blockable and preventable by dexamethasone treatment. Chronic footshock stress leads to a tolerance-like phenomenon, where no analgesia is seen after the treatment. This paradigm has served as the basis for studying the β-END/POMC changes which may occur acutely and chronically.

The approaches involve: 1) pulse-chase experiments in pituitary [anterior and intermediate lobe] in control, acutely stressed, and chronically stressed animals; 2) plasma studies quantifying β-END, β-LPH and POMC in the same animals, and assaying the ratio of acetylated versus nonacetylated products; 3) some brain studies using HPLC/RIA technique and multiple antisera to characterize changes in regional profiles upon stress.

The results to date suggest that all sizes of β-END-like immunoreactivity are releasable upon stress; that acute stress leads to an increase biosynthesis of β-END and a decrease in its half life in anterior lobe; that the opposite pattern, i.e. decreased rate of biosynthesis, is seen in the anterior lobe of chronically stressed rats; that the intermediate lobe is differentially altered chronically. Finally, the multiple forms of β-END in brain also exhibit significant changes.

The set of studies should: a) shed light on some of the mechanisms of stress-induced analgesia; b) provide a model for studying dynamics of regulation of POMC under acute and chronic physiological challenge.

60.3 ANALGESIA PRODUCED BY INTRATEHCAL ADMINISTRATION OF MORPHINE IS NOT ASSOCIATED WITH ALTERATION IN 5-HYDROXYTRYPTAMINE TURNOVER IN RAT SPINAL CORD. I.-H. Pang*, M. Vogt*, and M.R. Vasko. Dept. of Pharmacology, VA Medical Center and Univ. Texas Health Science Center, Dallas, TX 75235, Institute of Animal Physiol., Babraham, Cambridge, England CB2 4AT.

Evidence supports the hypothesis that analgesia produced by microinjection of morphine (M) into discrete brainstem nuclei involves activation of a serotonergic pathway descending from nucleus raphe magnus (NRM) into spinal cord. Intrathecal administration of M also produces antinociception but neurotransmitter correlates are not clearly established. The purpose of these studies is to determine if analgesia produced by superfusion of M onto the spinal cord is also associated with activation of 5-hydroxytryptamine (5-HT) containing neurons.

Male albino Wistar rats (250-350 g) were anesthetized and a metal guide cannula implanted into the skull directed at NRM or a polyethylene cannula (PE10) inserted into the subarachnoid space of the spinal cord and positioned with the tip in the area of T12 - L2. After 4-6 days, awake, unrestrained rats were administered M sulfate (calculated as base content) or sodium sulfate into NRM (10 µg in 0.5 µl) or onto spinal cord (10 or 50 µg in 10 µl). Analgesia was determined using both paw-pressure and tail-flick techniques. Ninety min after injection, rats were decapitated and brains and cords were homogenized in 0.1N HCl containing ascorbic acid. 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were extracted and assayed by fluorimetry. 5-HT turnover was estimated as the accumulation of 5-HIAA in both probenecid pretreated (200 mg/kg i.p.) and nonpretreated rats.

When microinjected into NRM, M caused significant analgesia (1.7-fold increase in pain threshold for at least 1 hr) and a statistically significant increase in 5-HIAA concentrations in posterior medullas and spinal cords of both probenecid pretreated (917 ± 32 to 1187 ± 62 and 540 ± 25 to 720 ± 34 ng/g, respectively) and nonpretreated (612 ± 50 to 877 ± 103 and 267 ± 38 to 352 ± 38 ng/g, respectively) rats. 5-HT levels were unaltered, indicating an increase in 5-HT turnover. Intrathecal injection of M (10 or 50 µg) produced profound analgesia (3-fold increase in pain threshold lasting 90 mins) but did not cause any significant change in 5-HT or 5-HIAA concentrations in posterior medulla and spinal cord of probenecid pretreated rats.

Thus although analgesia produced by M microinjection into NRM correlated with an increase in 5-HT turnover in NRM and spinal cord, no correlation was observed when M is administered intrathecally. These results do not support the suggestion that analgesia produced by intrathecal M is mediated by 5-HT containing neurons. (Supported by Veterans Administration, NIDA, and Medical Research Council, England)

60.4 THE EFFECT OF OPIATE PEPTIDES ON VASOPRESSIN RELEASE. C. D. Sladek, M. Gallagher and M. Mudd. Dept. of Neurology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

The role of opiate peptides in the control of vasopressin (VP) release was examined utilizing the organ-cultured rat hypothalamo-neurohypophyseal system (HNS). β-endorphin (10^{-5}M) significantly reduced unstimulated VP release by HNS explants on day 3 and 4 of culture ($p<.05$). This effect was not observed in the presence of naloxone ($5x10^{-5}$M). Further evidence for an inhibitory role of opiods on VP release was obtained in experiments in which explants were simultaneously exposed during the test hour on day 3 to the stable enkephalin analog, D-Ala-D-Leu-enkephalin (DADLE, 10^{-5}M), and either acetylcholine (Ach, 10^{-5}M), angiotensin II (AII, 10^{-5}M), or a 15 mosmol increment in osmolality achieved by adding NaCl. DADLE blocked stimulation of VP release by Ach, AII, and NaCl. These observations are consistent with the report that β-endorphin ($2x10^{-6}$M) and DADLE ($5x10^{-6}$M) attenuate electrically stimulated VP release from the neurohypophysis in vitro (Nature 284:350, 1980).

Inhibitory effects were not observed with other opiate peptides tested. Neither Leu-enkephalin (10^{-5}M) nor another stable enkephalin analog, D-Ala-Met-enkephalinamide (10^{-5}M) attenuated VP release stimulated by Ach, AII, or NaCl. Leu-enkephalin, added alone, caused an increase in VP release at concentrations of 10^{-7} to 10^{-5}M ($p<.005$) and did not significantly alter VP release at lower concentrations (10^{-10} or 10^{-9}M). Dynorphin also caused a concentration dependent increase in VP release. It was more potent than Leu-enkephalin with 10^{-10}M yielding a 217±42% increase in VP release ($p<.025$) and 10^{-6}M a 375±109% increase over control ($p<.01$). The effect of opiate antagonists on these stimulating actions of opiate peptides remains to be examined.

It is possible that multiple opiate receptor types are responsible for the diverse effects observed. Since the opiate peptides exhibit variable affinity for different receptor types, such a heterogeneous opiate receptor population could be differentially activated by individual opiate peptides. Furthermore, the receptor subtypes may be differentially localized within the hypothalamus or neural lobe such that, based on receptor affinity, the predominant site of action may differ amongst the opiates tested.

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60.5 PLASMA MEASURES OF BETA-ENDORPHIN-LIKE IMMUNOREACTIVITY IN DEPRESSIVES AND NORMAL SUBJECTS. J. Matthews,* H. Akil, J. Greden* S.J. Watson. (SPON: T. Morrow). Mental Health Res. Inst., Univ. of Mich., Ann Arbor, MI 48109.

The co-existence of ACTH and beta-endorphin (β-END) in the same pituitary cells has raised questions about the unique or combined role of these peptides in normal human physiology and in pathological states. One group of psychiatric patients which may have a defect in the regulation of the β-END/ACTH system are subjects suffering from endogenous depression.

The dexamethasone suppression test (DST) provides evidence for the presence of an abnormality in cortisol regulation in two-thirds of endogenously depressed patients. However, the mechanism underlying the faulty regulation of cortisol remains unknown, although it is likely to involve abnormal feedback control of the ACTH/β-END pituitary system. As a result there has been a great deal of interest in measuring β-END/β-LPH in the plasma of depressives, but these measures have turned out to be extremely difficult due to specificity problems and low circulating levels.

We will report on the development, validation and utilization of a very sensitive radioimmunoassay to plasma β-END. This has enabled us to examine the suppressibility of β-END/β-LPH, under dexamethasone challenge, in a population of depressed patients and normal controls. Contrary to a recent report, we have found a concomitant suppression of β-END/β-LPH in cortisol suppressors, which is consistent with out present understanding of the regulation of ACTH and β-END/β-LPH. However, the pattern of β-END suppression is somewhat different from that of cortisol suppression, suggesting that this may be a fruitful area for study. Chromatographic analysis of plasma β-END/β-LPH in cortisol suppressors and non-suppressors will be presented, as well as results on acetylated versus non-acetylated forms of β-END in the plasma of these subjects.

60.6 SELECTIVE OPIATE MODULATION OF HORMONAL AND PHYSIOLOGIC RESPONSES TO HEMORRHAGE. D.A. Bereiter, P.M. Plotsky and D.S. Gann. Brown Univ., RI Hospital, Providence, RI 02902

Selective nociceptor activation by tooth pulp nerve stimulation (TP) potentiates the ACTH response to moderate hemorrhage (H) in chloralose/urethane anesthetized cats. To assess the participation of endogenous opiates in this potentiation of ACTH, cats received naloxone (Nx, 100μg/kg,N) coincident with: a)H, 10ml/kg for 3 min, b)TP for 3 min (100-300μA, 0.2msec, 3Hz), or c)H+TP. Peripheral venous plasma samples were assayed for ACTH by RIA and for catecholamines (CA) by HPLC. TP potentiation of ACTH following H was not affected by Nx suggesting lack of endogenous opiate involvement in the apparent nociceptive-baroreceptive interactive facilitation of ACTH. In contrast, Nx greatly attenuated the fall in mean arterial pressure (MAP) and the rise in glucose following H or H+TP. The pressor effect of Nx on MAP could not be explained by altered CA release as both NE and E increased promptly to H or H+TP after Nx,equal to the CA response seen in untreated cats. The Nx attenuation of the glycemic response following H or H+TP was a consistent finding not significantly correlated with the diminished MAP response. To assess the possibility of a direct Nx effect on glucose release at the level of the liver, additional cats received intraportal injections of 20, 50 or 100μg/kg Nx. Neither of these Nx doses significantly altered the glucose or MAP response to H, suggesting that Nx is not acting at the level of the liver but rather is cleared rapidly there. We conclude that: a) the nociceptor potentiation of H-induced ACTH release does not depend on endogenous opiate participation, however b)endogenous opiate blockade by Nx selectively attenuates the MAP and glucose responses to H independent of effects on CA release. Supported in part by NIH grants AM 26831 and GM 27946.

60.7 OPIATE MEDIATED, STRESS INDUCED INCREASE IN THE INTAKE OF HIGH FAT DIET. K.K. Vaswani* and G.A. Tejwani* (SPON: J.R. Bianchine). Dept. of Pharmacology, Ohio State Univ. Coll. of Med., Columbus, OH 43210.

A number of studies on animals, including human beings, indicate that there is an increase in food intake due to acute stress. The main purpose of this study is to see whether or not acute stress induces a selective intake of a particular diet. Secondly, in view of the known role of endogenous opiate system in inducing feeding behavior, to determine its role in selective uptake of a particular diet. Male, Sprague Dawley rats (6-8 weeks old, Harlan) were given isocaloric control (standard lab chow), high carbohydrate, high protein and high fat diets (custom made diets, Bioserv Inc.). Following the subjection of animals to stress, food intake was monitored for 3 hours. Mild stress (food deprivation for 12, 24 and 48 hours) resulted in higher food intake in all the four groups. The effect was much greater in the group on high fat diet. Severe stress (food deprivation for 12 hours followed by forced swimming in water at 4°C for 10 minutes) also resulted in higher food intake in all of the four groups; control (154%), carbohydrate (174%), protein (310%) and fat (423%). When an opiate antagonist, naloxone (1 mg per kg body weight, i.p.) was given 1 hour before subjecting animals to severe stress, it reduced considerably the stress induced overeating in all the groups. For example, in animals given naloxone, severe stress induced increase in the uptake of diet was only; control (107%), carbohydrate (66%), protein (165%), and fat (87%). The ability of naloxone to reduce the intake of different diets was fat > protein > carbohydrate > control. These observations indicate that the food intake during acute stress may be mediated through the endogenous opiate system, which may be activated more by high fat diet than other diets. This may also suggest a possible involvement of the endogenous opiates in the diet induced obesity. (This work was supported by a grant from The Weight Watchers Foundation, Inc.).

60.8 OPIATE AND NONOPIATE SEIZURES INDUCED BY MORPHINE. H. Frenk and G. Urca. Departments of Psychology, and Pharmacology and Physiology, Tel Aviv University, Ramat Aviv, Israel.

The intraperitoneal (IP) administration of morphine hydrochloride at extremely high doses (300 mg/kg) produced analgesia, catalepsy, and electrographic spiking in rats that developed into electrographic seizure patterns after approximately 2.5 hours. Whereas naltrexone (12 mg/kg) reversed or blocked analgesia and catalepsy, and diminished electrographic spiking it precipitated electrographic seizure activity similar to that observed following IP morphine alone. These seizures were accompanied by behavioral convulsions. No tolerance to these seizures developed with repeated paired administration of morphine and naltrexone or in morphine tolerant rats, but rather potentiation was observed. Similarly, these seizures were potentiated in amygdaloid-kindled rats.

On the other hand, intracerebroventricular (ICV) injections of 100 μg of morphine induced electrographic seizures and spikes after approximately 3 min which could be blocked by naltrexone and did not occur in morphine tolerant rats.

It was concluded that morphine activates two different epileptogenic mechanisms, one mediated by opiate receptors, the other not.

60.9 CHRONIC INFUSION OF OPIATE PEPTIDES TO THE RAT CEREBRAL VENTRICLE WITH OSMOTIC MINIPUMPS: SUPRAEPENDYMAL CELL "IMMUNE-LIKE" RESPONSE. L.C. Saland, E. Ortiz* and A. Samora*. Dept. of Anatomy, Univ. of New Mexico, Sch. of Med., Albuquerque, NM 87131.

Earlier studies have shown that a single injection of beta endorphin to the lateral cerebral ventricle in rats will stimulate the appearance of macrophage-like supraependymal cells (Saland et al., '81, Soc. Neurosci. Abst. 7:95), suggestive of a "chemotactic" response to the peptide. Chronic infusions of opiate peptides to the brain will lead to analgesia and ultimate tolerance in rodents (Wei and Loh, '76). In this study, solutions of opiate peptides, naloxone or sterile saline, were infused to the lateral cerebral ventricle of adult male Sprague-Dawley rats, using previously implanted stainless steel cannulas attached via PE-60 tubing to Alza osmotic minipumps (Model 2001). Chronic infusions were made with ovine beta endorphin, met-enkephalin, alpha-endorphin (all 10^{-5}M) or sterile saline, for periods of 24 or 48 hours, during which animals remained undisturbed in home cages. At the end of the infusion periods, rats were lightly anesthetized with ether and perfused with 0.9% NaCl followed by a buffered aldehyde mixture. Brain tissue was prepared for electron microscopy. The supraependymal region above the median eminence in control (saline-infused) rats occasionally exhibited cells with extended cytoplasmic processes. In striking contrast, 24 or 48 hour beta endorphin infusion caused the appearance of numerous small (7-9 µm) rounded cells with short microvilli, which had the appearance of lymphocytes. These small cells were often associated with larger cells having extended processes and the morphologic characteristics of macrophages. Chronic infusion with alpha endorphin, naloxone, or met-enkephalin produced no major macrophage or lymphocyte-like cellular responses, although all three agents stimulated the appearance of numerous supraependymal neurons, which have been observed in other studies. These observations suggest that chronic infusion of beta endorphin will initiate an immune-like response within the cerebral ventricles, and correlates with recent in vitro findings of stimulation of cells of the human immune system (monocytes and lymphocytes) using opiate peptides (Van Epps and Saland, submitted).

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60.10 DYNORPHIN: CENTRAL CARDIOVASCULAR MODULATION IN DISCRETE BRAIN REGIONS OF ANESTHETIZED AND CONSCIOUS RATS. G. Feuerstein and A.I. Faden, Neurobiology Research Unit, Uniformed Services University, Bethesda, MD 20814

The presence of dynorphin (DY) in several brain regions known to modulate cardiovascular functions suggest that DY may play a role in central control of blood pressure (BP) and heart rate (HR). To investigate this possibility, DY (1-13, Penninsula) was injected into the lateral ventricle (icv, 10 µl) and hypothalamic nuclei (1.0 µl) of conscious, femoral artery cannulated rats (implanted under halothane anesthesia 36-48 hrs prior to experiment) and hypothalamic nuclei and the n. tractus solitarius (0.1 µl, NTS) of pentobarbitone anesthetized rats. Stereotaxic methods were used for guide cannula implantation in conscious rats and parenchymal injections (glass capillary 50 µm) in anesthetized rats. Cardiovascular responses were followed for 60 min; at the end of the experiments the brain was removed, frozen on dry ice, sliced (50 µm) on a cryostat, stained (0.1% thionine) and the site of injection confirmed microscopically; icv injections were confirmed by injections of dye. Vehicle injections (0.9% NaCl) had no cardiovascular effects by any route of injection. DY, icv, 6 and 60 nmol/300 g body weight had no effect on BP but elicited dose dependent increments in HR:+30±13 and +52±17 beats/min ($p < 0.01$, n=6-8). DY injected into the n. preopticus medialis (POM) of conscious rats (6-18 nmol) did not change BP, however HR was increased by +52±12 and +77±8 beats/min. DY, 6 nmol, injected into the POM and n. periventricularis hypothalami (HPV) of pentobarbitone anesthetized rats caused : hypotension, -23±1 and -22±6 mmHg in POM and HPV respectively ($p < 0.01$, n=6); bradycardia, -36±8 and -50±15 beats/min respectively ($p < 0.01$); and in the HPV only, DY also caused bradypnea, -23±7 respirations/min ($p < 0.01$). Naloxone (0.5 mg/kg, i.v.) injected at the peak of DY effect (hypothalamic injections in anesthetized rats) did not reverse the decrease in BP and only partially reversed the bradycardia; the decrease in respiration rate following DY injections into the HPV was completely reversed by naloxone. Injection of DY into the NTS (6 nmol) caused pressor response, +10.5±2.2 mmHg ($p < 0.01$) without change in HR or respiration rate; the pressor effect lasted for 10-15 min. These data suggest that DY may play a role in central cardiovascular control in both forebrain (diencephalic) and hindbrain (NTS) sites. Different cardiovascular responses were obtained in awake versus anesthetized animals emphasizing the importance of conscious animal studies in examining cardiovascular regulation.

60.11 LONG LASTING ANTICONVULSANT EFFECTS OF ENKEPHALIN MICROINJECTIONS. Zeev Elazar and Elias Motles. Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Israel.

Opioid peptides were shown to have a double action in the brain. Intracerebral administration induces seizures. On the other hand, naloxone studies have indicated a role for enkephalin in postictal behavioral depression (Holaday et al. Soc. Neurosci. Abst. 4,409, 1978; Frenk et al. Brain Res. 167, 435, 1979). Also, an anticonvulsive effect of opioid peptides was indicated (Tortella et al. Soc. Neurosci. Abst., 5, 542, 1979). We now report results of a study of the anticonvulsive effect using the electrical after-discharges model.

Cannulas with attached electrodes were lowered into the dorsal hippocampus in rats. Under urethane anesthesia, series of after-discharges (AD) were induced with threshold currents at intervals of 10-15 minutes, before and after enkephalin. Leu-enkephalin was infused (10-30µg in 0.5 - 1µl saline) into the dorsal hippocampus in the same area where stimulations and recordings were carried out. After enkephalin, the threshold of AD increased by 30-50 %. This increase in threshold was local, thresholds for contralateral ADs not being altered. This depressant effect was evident even when enkephalin did not have any obvious EEG effect, but was much stronger after the end of an enkephalin produced epileptiform sequence. Also, a second enkephalin infusion four hours after the initial one, induced an additional increase in threshold. Naloxone (0.5 - 1mg /kg i.v.) given after the end of the enkephalin epileptiform sequence abolished the depressant effect and restored the pre-enkephalin thresholds.

Ach + neostigmine infused in the same way as enkephalin, induced fast rhythmic waves and spikes. The AD threshold was increased for 10-20 min after the Ach epileptiform sequence. By comparison, the depressant effect of enkephalin was much longer, lasting 1-5 hrs.

Repeated convulsions were shown to produce long lasting elevation in the enkephalin levels (Hong et al. Brain Res. 177, 273, 1979). Our present data suggest a long lasting elevation of the enkephalin content produced by the injected enkephalin or by some mechanism related to its excitatory effect. In favor of this suggestion, is the finding that naloxone abolishes the depressant effect. This long lasting anticonvulsant effect could explain the tolerance shown to the epileptogenic effect of enkephalin (Elazar et al. Life Sci. 24, 541, 1979).

60.12 PHARMACOLOGICAL EVIDENCE FOR CHANGES IN MU AND KAPPA OPIATE RECEPTORS FOLLOWING AMYGDALOID KINDLING. A. Mansour & E.S. Valenstein. Dept. Psych. & Neurosci. Lab., Univ. Mich., Ann Arbor, MI, 48109.

We have shown that amygdala kindling produces lasting changes in morphine sensitivity. Kindled mice showed clonic convulsions, more stereotyped running and an enhanced Straub tail following morphine (Mansour et al, Physiol. & Behav., 1981, 27, 1117). Kindled rats also show convulsions following morphine (Frenk, pers. comm.). To test the specificity of these opiate effects, we compared the response of kindled mice to morphine, a mu receptor agonist, and ethylketazacine (EKC), a kappa agonist.

C57BL/6 mice were implanted with amygdala electrodes and stimulated daily (1 sec., 50 uAmp, 60 hz) to a criterion of 7 consecutive Stage 5 convulsions and tested 3 days later. Control animals were implanted and handled, but not stimulated.

Experiment I. Dose-response curve for morphine and EKC convulsions in kindled animals. Four kindled groups (6-9/group) received morphine (5, 10, 17, or 25 mg/kg, i.p.) while another 5 kindled groups received EKC (0.3, 1.0, 1.7, 3.0 or 10 mg/kg,i.p.). EKC (1.7, 10.0, 30.0 or 100.0 mg/kg, i.p.) was given to 4 control groups. Percentage of mice showing convulsions was recorded for 30 or 100 minutes following EKC or morphine, respectively. A morphine dose-response curve was not performed with control mice, since C57's don't show clonic convulsions even after lethal doses.

None of the kindled mice convulsed at 5, 40% convulsed at 10, 66% convulsed at 17, and 89% convulsed at 25 mg/kg morphine. Similarly, no kindled animal convulsed at 0.3, 25% convulsed at 1.0, 88% convulsed at 1.7, and 75% convulsed at 3.0 and 10 mg/kg EKC. No control mouse convulsed at 1.7, 16% convulsed at 10.0 and 30.0 mg/kg and none convulsed at the lethal dose of 100 mg/kg EKC.

Experiment II. Naloxone antagonism of morphine and EKC convulsions. 4 groups were injected with naloxone (0.001, 0.01, 1.0, or 5.0 mg/kg, i.p.) 5 min. prior to 25 mg/kg morphine, while 3 groups received naloxone (0.001, 0.01, or 0.1 mg/kg, i.p.) before 1.7 EKC. Morphine and EKC doses were equally effective in producing convulsions in kindled mice. Results: 0.1 mg/kg naloxone block EKC convulsions; while 0.01 mg/kg was equally effective with morphine.

Summary: Naloxone can block the dose-dependent convulsions induced by morphine and EKC in kindled animals. The higher doses of naloxone needed to antagonize EKC convulsions are consistent with the view that more than one type of opiate receptor is changed by kindling. Responsiveness to all convulsants is not increased as kindled mice are not more sensitive to strychnine (unpublished). As EKC has been reported not to produce catecholamine or cholinergic dependent effects (Wood et al, JPET, 1980, 215, 697) these transmitters may not be involved in the present results.

60.13 A PHARMACOLOGICAL SYSTEM FOR THE CHARACTERIZATION OF SUBCLASSES OF OPIOIDS. K.A.Bonnet, M. Orbuch*, Dept.Psychiatry, New York University Sch. of Med., New York, N.Y. 10016

Several years ago we reported a new standardization of the jump-flinch technique that utilized still-frame analysis of animal responses to footshock for the specification of particular types of responses and the correlation of each with levels of inescapable footshock. Intracerebral administration of opioid agonists of several types acutely , or chronically have provided anatomical levels at which specific response types are elicited or mediated. The procedure has been developed through special aparatus design to permit the recognition of specific response types by computer algorithm, and the titration of thresholds for four motor response types and for vocalization.

Specific vocalization and motor thresholds for each of several of opioid and nonopioid analgetic compounds indicates specific profiles and time courses that allow discrimination between classes of compounds, and between subclasses of opioids with high sensitivity and with excellent reproducibility.

Specific compounds of the mu, kappa and sigma opioid agonist subclasses are easily distinguished by the time course and the pattern of effects on thresholds for the various response types. Kappa compounds, now presumed to be identified with dynorphin-preferring forebrain sites, were unique in producing long-lasting elevation of thresholds for all response types in the same proportion, whereas sigma and other hallucinogenic compounds elevated a "type three" response category selectively and the mu agonists elevated a "type two" response category preferentially. Selective alteration of response profile permitted discrimination between aspirin compounds, narcotics and psychotropic compounds that also produce analgetic effects of some types, such as delta-9-tetrahydrocannabinol, and was highly sensitive to specific anatomical brain regions at which some of these compounds appear to act through specific receptor subtypes selectively.

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60.14 HYPOTENSIVE EFFECT OF MORPHINE MICROINJECTED INTO THE MEDULLARY RETICULAR FORMATION OF THE CAT. D. L. Wolf and J. S. Mohrland, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Hypotension can occur following morphine administration in man, an effect believed to result from a peripheral release of histamine and a CNS action to decrease adrenergic tone. A medullary site for the CNS action was suggested by studies in which morphine was injected intracisternally, or fentanyl was microinjected into the n. ambiguus (NA). The present study used the microinjection technique to identify sites in the medulla at which morphine elicits hypotension.

Fourteen cats of either sex were anesthetized with halothane, pentobarbital (38 mg/kg, i.p.) or a midcollicular lesion plus Flaxedil. The posterior cranium was removed and the floor of the IVth ventricle exposed. The femoral artery and vein were cannulated for blood pressure measurement and drug injection, respectively. Heart rate (HR) was monitored from the blood pressure signal by a tachograph or from fast-speed segments of the polygraph trace. Morphine sulfate (5-10 µg free base) was microinjected unilaterally at 59 sites in the medulla from P8 to P14 (posterior to the interaural plane). The location of the microinjection sites was verified histologically by the deposition of fast green dye. Changes in mean arterial pressure (MAP) of less than 10 mmHg were considered to have no effect.

Most responses of decreased MAP were elicited from microinjections of morphine into the lateral reticular formation, including the n. reticularis lateralis (NRL) and NA, from P9 to P13. Of the lateral reticular formation sites, 22/39 showed a decreased response, 5/39 showed an increased response, and 12/39 had no effect on MAP. The decrease ($\bar{X} \pm$ S.D.) in MAP for 22/39 sites was 16.9± 7.7 mmHg with a range of 10 to 34 mmHg. The mean change in MAP following morphine was significantly different ($p<0.001$) from that following CSF microinjected at the same sites. Naloxone (0.5-2.0 mg/kg, i.v.) at least partially antagonized the morphine hypotensive effect in 9/15 cases. HR was not consistently altered; decreased MAP was accompanied by increased HR in 6/14 cases, decreased HR in 5/14 cases, and no change in HR in 3/14 cases. There was no difference in response between the various anesthetic modes.

The NA and NRL have been implicated in central cardiovascular control. Other studies have shown that cells in these nuclei are primarily inhibited by opioid drugs. Collectively these findings suggest that the reduction in MAP following morphine administration results, at least in part, from an inhibition of neuronal activity in the NA and NRL with a resultant decrease in sympathetic outflow. Furthermore, the present study suggests that bradycardia may accompany, but is not essential to the production of, the hypotensive episode following morphine microinjection.

60.15 DIRECT COMPARISON OF THE ANTINOCICEPTIVE EFFECTS OF CAPTOPRIL AND THIORPHAN IN MICE: W.L. Autry*, B.S. Barbaz* and W.D. Cash* (Spon: J.A. Norman). Res. & Dev. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

The antinociceptive effects of captopril (CP) (1), an angiotensin-converting enzyme (ACE) inhibitor, and thiorphan (TP) (2), an inhibitor of enkephalinase, were assessed in mice using the hot plate (55° C) test (2). Jump latencies were measured 5, 15 or 30 min after either intracerebroventricular (ICV) or intravenous (IV) drug administration. Each trial was terminated after the first jump response, or after 240 sec if no response occurred. By the ICV route, both CP and TP elevated jump latencies significantly at 5 and 15 min. The lowest effective dose of CP was 300 µg (in 10 µl of distilled water) at both time periods. The effective dose range for TP at 5 min was 7.5 to 60 µg (in 10 µl of distilled water), while at 15 min the dose range was 1.88 to 60 µg. These treatments typically elevated latencies approximately 2-fold above those following vehicle treatment. Thirty minutes following ICV administration, CP had lost its effectiveness but TP was active at 60 µg. Following IV administration, CP (10-300 mg/kg) and TP (7.5-100 mg/kg) significantly increased jump latencies assessed 5 and/or 15 min later. Although CP (300 mg/kg IV) was still active 30 min later, TP (100 mg/kg IV) had no effect. The analgesic effects of both CP and TP were antagonized by pretreatment with naloxone (1-2 mg/kg s.c.) 5 min prior to IV or ICV injection of either enzyme inhibitor. When ineffective doses of CP (100 µg ICV) or TP (1 or 3.75 µg ICV) were concomitantly administered with an ineffective dose of met-enkephalin (20 µg ICV), significant increases in jump latencies were observed at 5 and/or 15 min. In confirmation of previous reports (1, 2), these data indicate that both CP and TP produce analgesia which is, at least in part, mediated by endogenous opiate substrates in the central nervous system. Our direct comparison of the two drugs by the ICV route shows that TP is considerably more potent and longer acting than CP. TP probably elicits analgesia by preventing the breakdown of enkephalin by enkephalinase (2). CP has weak inhibitory activity towards this enzyme (2, 3), which might account for its analgesic property. However, the possibility also exists that the analgesic action of CP involves inhibition of ACE, which cleaves arg-phe from the opioid heptapeptide met-enkephalin-arg^6-phe^7 (4). Studies with new generations of ACE inhibitors might clarify the mechanism underlying the analgesic activity of CP.

(1) Stine, S.M. et al.: Brain Res. 188:295, 1980. (2) Roques, B.P. et al.: Nature 288:286, 1980. (3) Hudgin, R.L. et al.: Life Sci. 29:2593, 1981. (4) Benuck, M. et al.: Biochem. Biophys. Res. Commun. 99:630, 1981.

60.16 DIFFERENTIAL EFFECTS OF INTRATHECAL MORPHINE, ETHYLKETOCYCLAZOCINE (EKC) AND NALBUPHINE ON THE HOT PLATE AND WRITHING RESPONSE. C. Schmauss*, C. Doherty* and T.L. Yaksh* (SPON: J.P. Whisnant) Mayo Clinic, Rochester, MN 55905.

We have systematically examined the effects on the hot plate response latency and acetic acid evoked writhing of intrathecally administered agents thought to be putative ligands for the µ (M: morphine); κ (EKC: ethylketocyclazocine); δ (DADL: d-ala^2-d-leu^5-enkephalin); and σ (SKF: SKF-10047) receptors, as well as a partial agonist nalbuphine. Rats were implanted with intrathecal catheters. After recovery, injections of 10 µl of drug in saline vehicle followed by 10 µl of saline were made and the latency to licking of the hind paw was assessed on the 52.5°C hot plate. Mean response latency in this test under control conditions was 16 ± 0.3 sec. Cut-off time in the absence of a response was 60 sec. The writhing response was evoked by the i.p. administration of 0.5 cc/300 gr of 10% acetic acid. In each writhing experiment, 6 intrathecal catheter-implanted animals were injected with drug and 6 with saline. The magnitude of the writhing response was noted on a scale of 0-3 at 5-min intervals during the following 50 min by an observer blind as to the drug treatment received by each of the 12 animals. The writhing score for each experiment was expressed as a percent of the animals receiving intrathecal saline. The intrathecal ED_{50} (nmoles) of each agent on the hot plate and writhing test are presented below.

	Hot Plate	Writhing
Morphine	6.0	1.1
Nalbuphine	>560	38
EKC	40.5	75.9
DADL	10.3	>320
SKF-10047	>390	>390

As can be seen, on hot plate, the relative potency was morphine > DADL >> EKC >>> nalbuphine = SKF = 0, while on the writhing test, the potency was morphine > nalbuphine > EKC >>> DADL = SKF = 0. At the ED_{50} doses, no effect on motor function was observed with any drug. The effects of all of the active agents were significantly antagonized by the co-administration of naloxone (1 mg/kg, s.c.), a treatment which alone had no effect on the magnitude of either response. To the extent that the respective agents represent selective ligands for the respectively designated opioid receptor populations, the different structure activity relationship observed between the two tests strongly suggests the likelihood that different subpopulations of naloxone-sensitive receptors may mediate the alterations produced by spinally acting drugs in the rostral transmission of nociceptive information evoked by thermal and visceral stimuli. (Spon. by Mayo Fndn. and DA-02110.)

60.17 INVOLVEMENT OF ENDORPHINS IN ANAPHYLACTIC SHOCK IN MICE, S. AMIR and M. Harel. Dept., Isotope Res., Weizmann Institute of Science, Rehovot, Israel.

Endorphins are implicated in the pathophysiology of endotoxic hemorrhagic and spinal shock since apparent blockade of their receptors by the specific opiate antagonist, naloxone, can improve cardiorespiratory parameters as well as survival in these shock states. It is suggested that the stress of shock promotes the release of endorphins, which subsequently depress respiratory and cardiovascular activity, thereby contributing to the lethal effect of shock. The present study demonstrates involvement of endorphins in anaphylactic shock, an allergic condition associated with circulatory collapse, resulting from an antigen-antibody reaction that takes place all through the body after an antigen to which the organism is sensitive has entered the circulatory system. Male ICR mice were sensitized to bovin serum albumin (BSA) by an i.p. injection of 2 mg protein in 0.2 ml aluminium hydroxide adjuvant. Anaphylactic shock leading to death in over 80% of animals was induced by an i.v. injection of 25 ug BSA in 0.2 ml saline 10 days following sesitization. Administration of naloxone (10 mg/kg) or naltrexone (1 mg/kg) prior to induction of shock decreased anaphylactic death by 70 and 68%, respectively ($P<0.01$). Pretreatment with methyl naltrexone, a quaternary analog of naltrexone which is impermeable to the blood brain barrier (1-5mg/kg), did not alter mortality rate. Also, naloxone and naltrexone, but not methyl naltrexone, protected against anaphylactic death when administered concomitantly with the shocking dose of BSA or 10 min after injection of the antigen. These results suggest involvement of endorphins in the pathophysiology of terminal anaphylaxis in mice. Also, they imlicate the brain opiate receptors in this effect. To investigate the possibility that endorphins contribute to terminal anaphylaxis by blocking central sympathetic discharge, mice were treated with chlorisondamine (5 mg/kg), a ganglionic blocking agent, 2 h prior to induction of shock. Ganglionic blockade, which inhibits sympathoadrenal medullary response to stress increased mortality in control mice (from 80 to 90%) and significantly diminished the beneficial effect of naloxone (10 mg/kg i.v.) in anaphylaxis. It is suggested that opiate antagonists protect against anaphylactic death by increasing sympathetic compensatory processes, which may be blocked by endorphins. The possible use of specific opiate antagonists and neuropeptides with anti endorphin effects, e.g. TRH, MIF-1, in anaphylactic shock is discussed.

60.18 THE ROLE OF ENKEPHALINS IN THE SACRAL PARASYMPATHETIC REFLEX PATHWAYS TO THE URINARY BLADDER OF THE CAT. T. Hisamitsu,* B.P. Roques,* and W.C. deGroat. Dept. Pharmacol., Univ. Pittsburgh, Pittsburgh, PA 15261; Dept. Physiol., Showa Univ., Tokyo, Japan; Dept. Pharm et. Biol., R. Descartes Univ., Paris, France.

In the cat leucine enkephalin (L-Enk) has been demonstrated immunohistochemically in preganglionic neurons and in nerve terminals in the lateral band of the sacral parasympathetic nucleus, an area which is involved in the control of the urinary bladder. The present pharmacological experiments were undertaken to examine the function of sacral enkephalinergic mechanisms in bladder reflexes. In cats anesthetized with chloralose, bladder activity was monitored by recording intravesical pressure via a urethral cannula. Activity in efferent pathways to the bladder was also recorded from postganglionic parasympathetic nerves on the bladder surface, and in sympathetic postganglionic axons in the hypogastric nerve. Vesical afferent axons were stimulated by rectangular pulses applied to electrodes on the pelvic nerve. Drugs were applied intrathecally at the level of the sacral segments through a polyethylene tube. The intrathecal administration of leucine-or methionine enkephalin (L-Enk, M-Enk) 30-300 µg in a volume of 0.05 ml produced weak and short lasting (3-30 min) depressions of spontaneous bladder contractions and vesical postganglionic firing. The inhibition was detectable within 1-2 min following the injection. Enkephalin analogs (D-alanine leucine enkephalinamide and D-alanine methionine enkephalinamide) which are more slowly metabolized produced more prominent inhibitory effects in threshold doses ranging from 3-30µg. The inhibitory effects were dose dependent and varied from a reduction in amplitude and frequency of bladder activity to a complete suppression of reflex responses. The inhibition persisted for 1.5-3.5 hours. Enkephalins did not block reflex firing on the hypogastric nerve in response to stimulation of pelvic nerve afferents. Naloxone (3-30µg) reversed the inhibition elicited by the enkephalins within 1-5 min after administration. Large doses of naloxone (30-100µg) increased the frequency of bladder contractions or produced a tonic contraction of the bladder and a tonic discharge on the vesical postganglionic nerves. These responses persisted for 0.5-2 hours. The injection of thiorphan (10-100µg) an enkephalinase inhibitor enhanced the inhibitory effects of L-Enk and in some animals produced a direct and prolonged (0.5-3 hours) depression of bladder reflexes. The direct effects of thiorphan were antagonized by naloxone (3-60µg). These data coupled with other findings showing that naloxone stimulates bladder reflexes in untreated cats suggests that the sacral outflow to the bladder is subject to a tonic enkephalinergic inhibitory control.

60.19 OPIOID, NORADRENERGIC AND ANDROGEN INTERACTIONS IN THE REGULATION OF LUTROPIN (LH) IN THE MALE RAT. J. Ellingboe, S.L. Garber*, A. S.T. Skupny* and H.R. Schroeder*. Alcohol & Drug Abuse Research Center, Harvard Medical School-McLean Hospital, Belmont, MA 02178.

Opiate drugs and endogenous opioids inhibit, while the opiate antagonists naloxone (NX) and naltrexone (NTX) enhance, LH release in man and rat (Mirin, S.M. et al, Psychoneuroendocrinology 1:359, 1976; Cicero, T.J. et al, J. Pharmacol. Exp. Ther. 201:76, 1977). Androgens suppress, and castration increases plasma LH, with effects on pulse amplitude and frequency similar to those of opiate agonists and antagonists. Noradrenergic systems appear to drive LH release by acting at hypothalamic sites that control luliberin (LRF) secretion.

Studies of opioid involvement in LH regulation were carried out with long-term castrated male rats (400-500 g), catheterized by jugular vein cannulae to permit 4-h serial blood sampling at 10-min intervals in unanesthetized animals. Some rats were pretreated with testosterone propionate (TP)(0.1-10 mg/kg in oil, s.c.), with reserpine (R)(0.5 mg/kg, i.p.), or by s.c. implantation of NTX-containing Alzet minipumps (dose rate = 1.25 mg/kg/day).

Control castrate plasma LH levels were 395±194 ng/ml. Morphine sulfate (MS)(10 mg/kg, s.c.) decreased LH significantly 30-160 min after injection. NTX infusion for 2-5 days increased LH 60%. All doses of TP lowered LH significantly on the day after administration. Clonidine (CL)(0.5 mg/kg, s.c.) induced a 10-fold increase in plasma LH in R- and MS-treated rats, but did not stimulate LH release in the TP-treated rats. MS did not alter the LH response to CL in R-treated rats. Alpha-adrenergic antagonists blocked NX- and NTX-induced increases in LH. NX (10 mg/kg, s.c.) elevated plasma LH in control castrated rats, but had no effect on LH in TP-treated rats. Conversely, TP was just as effective in lowering LH in NTX pump implanted rats as in controls.

These results indicate that TP blocks the LH-stimulatory effect of opioid antagonists in a noncompetitive manner and that opiate antagonists cannot reverse the inhibitory effect of androgens on plasma LH, suggesting that androgens act at neuronal loci that are sequentially between the sites of opioid control and LRF secretion. The findings also indicate that opioids act at the level of noradrenergic neurons (or at higher sites), rather than at LRF neurons, and that androgen negative feedback regulation of LRF secretion occurs after noradrenergic input. Because different results have been obtained in studies with short-term castrated rats it must be emphasized that these tentative conclusions apply only to long-term castrates. Our findings are consistent with those of Kalra and Simpkins (Endocrinology 109:776, 1981) who also suggest indirect opioid inhibition of LH secretion, via modulation of noradrenergic input to LRF neurons.

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61.1 BEHAVIORAL AND ELECTROPHYSIOLOGICAL PROFILE OF DYNORPHIN-(1-17) AND ANLAOGS SUGGESTS BIOLOGICALLY ACTIVE NON-OPIATE COMPONENT. J.M. Walker*, D.H. Coy*, and H. Akil (SPON: S. Berent). Mental Health Res. Inst., Univ. of Mich., Ann Arbor, MI 48109

Early studies of dynorphin-(1-13) showed little or no analgesia and a profile of other behavioral changes quite different from that of the other endogenous opiates. For example, a number of the motor and behavioral changes could not be antagonised by naloxone. However, these effects were difficult to interpret because the full sequence of dynorphin was unknown.

The present studies indicate that microinjection of the full dynorphin-(1-17) molecule (5 to 20 µg intracerbroventricularly) produce marked EEG seizures in the cortex and flattening of the hippocampal EEG. These effects were sometimes accompanied by strong motor changes similar to those observed with dynorphin-(1-13). However, the motor changes were sometimes present when the EEG appeared relatively normal.

Pain sensitivity appears relatively unaffected by dynorphin in that even at 20 µg, a dose that produces seizures in virtually all rats, no changes in tail-flick latency occur. Dynorphin-(1-17) also fails to induce changes in heart-rate.

As with dynorphin-(1-13) pretreatment with even high (20mg/kg) doses of naloxone fails to prevent the occurrence of seizures and motor changes. Preliminary data further suggest that Des-Tyr-dynorphin, a fragment having virtually no affinity for opiate receptors, produces similar effects. These data suggest beyond its opiate effects, a second active non-opiate sequence exists within the dynorphin core. (See Moises et al, this meeting).

61.2 ELECTROPHYSIOLOGICAL ACTIONS OF DYNORPHIN AND DES-TYROSINE-DYNORPHIN SUGGEST POTENT NON-OPIATE EFFECTS. H.C. Moises, J.M. Walker*+, D.H. Coy*++, S.J. Watson+ and H. Akil+. Dept. of Physiology and +Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109; ++Vet. Admin. Hosp., New Orleans, LA 70140.

Dynorphin produces a pattern of behavioral and motor effects which distinguishes its actions from those of other opiate-like peptides. Many of its effects are not reversed by the opiate antagonist naloxone, raising the possibility that some active site in the dynorphin sequence may produce effects that are not opiate in nature. In the present study, we examined the electrophysiological actions of dynorphin $_{1-17}$ (DYN) on the activity of single neurons in the hippocampus and parietal cortex of the rat. These experiments were prompted by our recent findings of significant amounts of DYN immunoreactivity in these brain regions (see Watson et al., this meeting) and because much is already known about the electrophysiological actions of opiates and opioid peptides in these structures.

Five-barreled glass micropipets (3-8MΩ) were used to record extracellularly from single hippocampal pyramidal and cerebrocortical neurons and to apply peptides at the site of recording by iontophoresis or pressure ejection. Brief (10-30 sec) applications of DYN (4-8mM) produced a dose-dependent inhibition of both spontaneous discharge and glutamate evoked firing in 18 of 24 (CA1) and 18 of 21 (CA3) pyramidal neurons. The inhibitory effect of DYN typically had a brief latency to onset, showed little evidence of tachyphylaxis and was produced equally well both by pressure and iontophoretic ejection of the peptide. In contrast to the effects produced by enkephalin, excitatory responses to DYN application were rarely observed (2 of 45 cases). In tests on 8 cells, iontophoretic application of naloxone failed to prevent (3 of 3 neurons) or reverse (5 of 5 neurons) DYN induced inhibitions. Moreover, in tests on the same populations of cells, iontophoretic or pressure ejection of the opiate inactive fragment des-tyrosine dynorphin (dT-DYN) produced comparable inhibitory effects on neuronal activity in both CA1 (5 of 7 cells) and CA3 (4 of 5 cells). A similar pattern of inhibitory effects was also observed with both DYN and dT-DYN in all 8 cerebrocortical neurons tested.

In summary, the actions of DYN in hippocampus appear to differ significantly from those of the classical opiates in that inhibition was routinely produced, naloxone failed to affect the response, and an opiate inactive DYN fragment produced the same effects as the parent compound. The possibility raised here is that a second, non-opiate site with potent biological activity may occur within the DYN sequence.

61.3 RESPONSE OF RAT HIPPOCAMPAL NEURONS TO ELECTROPHORETICALLY AND PNEUMATICALLY APPLIED DYNORPHIN AND OTHER OPIOID PEPTIDES. S.J. Henriksen, G. Chouvet*, and F.E. Bloom. Arthur V. Davis Center, The Salk Institute, San Diego, CA, U.S.A. and *INSERM U171, University Claude Bernard, Lyon, France

Recent immunohistochemical and radioimmunochemical investigations have demonstrated a differential distribution of immunoreactive dynorphin (DYN17) in rat brain (see McGinty et al., Chavkin et al. this volume). Because of the existence of prominent DYN17 immunoreactivity in a major intrinsic fiber system within the rat hippocampus, the mossy fiber system, we have examined the possible functional role of DYN17 in this structure. Spontaneous and evoked single cell activity and field potentials have been recorded from the CA1-CA3 cellular fields in halothane or urethane anesthetized rats. DYN17 (3-5 mM in saline), Dynorphin [(1-13, 1-17); 0.3-5 mM in saline], leu-enkephalin (5-10 mM in saline), morphine (5-10 mM) ethylketocyclazocine (5-10 mM in H_2O), Naloxone HCl, (5 mM in H_2O), monosodium glutamate (0.5 M in H_2O), $Mg^{++}Cl$ (1 M in H_2O), and bicuculline (13 mM in saline) have been electrophoretically or pneumatically applied to single CA1-CA3 neurons, and changes in spontaneous discharge of these neurons have been monitored before, during, and after application of these drugs (alone, or in combination). As has been previously observed for enkephalins and their derivatives, DYN17 and DYN13 had a predominantly excitatory effect on most CA1-CA3 neurons (70%) in halothane anesthetized rats. This effect was characterized (particularly in CA3) by a slow onset and prolonged excitatory effect. This response could be blocked by naloxone or by co-administration of Mg^{++} ion, suggesting an indirect (synaptic) mechanism of excitation similar to that hypothetized for enkephalin. However, a significant number of CA3 neurons (12%) exhibited a non-naloxone sensitive inhibitory response that was time locked to peptide application. Field potential analysis of CA1-CA3 neuronal responses to mossy fiber activation also indicated an excitatory, Mg^{++} reversible, action of iontophoretically applied DYN17. These preliminary observations of the naloxone-reversible excitations and naloxone-insensitive inhibition by dynorphin on hippocampal CA3 neurons support our cytochemical and assay studies showing diverse opioid systems within the rat hippocampus. In addition, these functional studies are congruent with other evidence suggesting multiple opioid mechanisms in this structure. Supported by DA 01785, the Klingenstein Foundation, INSERM and Fondation de l'Industrie Pharmaceutique pour la Recherche.

61.4 ACTIVATION OF LOCUS COERULEUS NEURONS IN RAT BY UM 1046, A DRUG THAT MIMICS OPIATE WITHDRAWAL IN NORMAL ANIMALS. R.J. Valentino* and G. Aston-Jones, The Salk Institute, San Diego, CA 92138 (Spon: S.L. Foote)

UM 1046 (N- cyclopropylmethyl -1-2-3-4-5-6 -hexahydro-8 -hydroxy-6-methyl -3-benzazocine), when administered to narcotic-naive monkeys produces behavioral signs similar to those observed during antagonist-precipitated morphine withdrawal. In addition, UM 1046 (1.0 - 5.6 mg/kg, IV) in naive rats produces a syndrome consisting of retching, tremor, wet dog shakes, rhinorrhea, lacrimation, salivation, teeth chattering, chewing and abnormal postures that subsides in 15-20 min. Other drugs including certain phosphodiesterase inhibitors also produce this "quasi-morphine withdrawal syndrome" (QMWS). Because increased activity of noradrenergic locus coeruleus (NE-LC) neurons has been reported for both antagonist-precipitated narcotic withdrawal and QMWS produced by methylxanthines (Aghajanian, 1978; Grant and Redmond, 1981), it was of interest to determine whether UM 1046 had a similar effect on these neurons. Spontaneous discharge rates of NE-LC neurons in halothane-anesthetized rats were recorded before and after IV injections of UM 1046 (1.0-5.6 mg/kg). UM 1046 produced a rapid (10-30 sec latency), dose-dependent increase in the firing rate of NE-LC neurons that reached a maximum of about 300% of the pre-drug rate and returned to the control rate about 20 min after injection; these effects were not related to the baseline spontaneous control rate of the cells. A comparison of cumulative and single-dose response curves revealed that desensitization occurs to this drug-induced increase in NE-LC discharge. Stimulation of muscarinic receptors is integrally involved in the response to this drug since UM 1046 (5.6 mg/kg) was ineffective after the IV administration of scopolamine (0.5 mg/kg). In contrast, methylscopolamine (1.0 mg/kg), which does not penetrate the blood brain barrier, had no antagonist action against this dose of UM 1046, indicating that the increase in spontaneous rate of LC neurons produced by UM 1046 requires activation of central muscarinic systems. This study complements previous work suggesting that increased activity of NE-LC neurons is associated with withdrawal-like behaviors. In addition, the results support findings using the guinea-pig ileum and drug discrimination procedures that a cholinergic link is essential for the QMWS produced by the benzazocine. This study was supported by U.S.P.H.S. grants DA 05194, DA 01785, and AA 07273.

61.5 LOSS OF AGONIST RESPONSE IN THE HIPPOCAMPAL SLICE DURING PROLONGED INCUBATION WITH OPIOID PEPTIDES. M.E. King*, R.J. Valentino*, E. Bostock, and R. Dingledine (SPON: J.E.Wilson). Dept. Pharmacol., Univ. N. Carolina, Chapel Hill, NC 27514.

Opiates and opioid peptides potentiate the synaptic activation of CA1 pyramidal neurons in the rat hippocampal slice. This opioid effect can be quantitated as a concentration dependent shift to the left in the input-output (I/O) curve constructed by plotting the population spike as a function of the field EPSP. We investigated the timecourse of this effect. I/O curves (30-100 points each) were constructed in individual slices with the aid of an LSI-11 computer every 3-10 min for 5-7 hours. After a 1.5-2 hr control period, a 5 hr perfusion with morphine (20 µM) or [D-ala,D-leu]-enkephalin (DADL, 1 µM), or a 2.5 hr perfusion with morphiceptin (10 µM), was initiated. The maximum effect produced by all three agonists was the same, and the peak effect was obtained within 12-25 min in each case. However, the effect of both opioid peptides declined during constant perfusion. Approximately 60% of the peak effect was lost after 1 hr incubation in morphiceptin, or after 4 hr in DADL. In contrast, the response of the tissue to morphine gradually increased over a 4 hr incubation. Following perfusion of the slices for 4 hr in DADL or for 1.5 hr in morphiceptin, 1 µM naloxone returned the I/O curve to its pre-drug control position, reversing the residual agonist effects. No "overshoot" of the I/O curve was observed, suggesting that dependence had not developed. Thus, a form of desensitization to opioid peptides, but not to morphine, develops within hours in the hippocampus. This loss of response to the peptides is not likely due to proteolysis since the peptides were constantly replenished by perfusion. The response loss was observed with both mu-specific (morphiceptin) and delta-specific (DADL) opioid agonists, and thus may not be a property of any single subtype of opioid receptor. The rather gradual loss of response may reflect a differential interaction of opioid peptides and morphine with their receptors in the hippocampus. Supported in part by the Sloan Foundation and NIDA DA-02360.

61.6 EFFECTS OF MICROINJECTION OF OPIOIDS INTO AND ELECTRICAL STIMULATION (ES) OF THE CANINE PERIAQUEDUCTAL GRAY (PAG) ON EEG ELECTROGENESIS (EEG), HEART RATE (HR), PUPIL DIAMETER (PD), BEHAVIOR AND ANALGESIA. J.G. Wettstein*, S.G. Kamerling and W.R. Martin, Dept. of Pharmacology, Univ. of KY, Lexington, KY 40536.

The present study was conducted in order to come to a better understanding of the physiologic processes of the PAG and how such processes may be modulated by opioid systems. Chronic guide cannulae were stereotaxically placed above the caudal PAG in female beagle type dogs. Microinjection cannulae (30 G) or concentric, bipolar stimulating electrodes (26 G) were lowered below the guide cannula into the PAG. All microinjected drugs were delivered i.c. in 1.0 µl volumes over 1 min. Dogs received PAG ES or microinjections of fentanyl (FEN; 1,3,9 µg i.c.), morphine (MOR; 57 µg i.c.), ethylketazocine (EKC; 1.7,5,15 µg i.c.), nicotine (5,20 µg i.c.) or saline (i.c.). The opioid antagonist naltrexone (NTX) was administered (2 mg/kg i.v.) alone and 20 min prior to FEN (9 µg i.c.). ES consisted of a 45 sec train of square waves (200-400 µA, 0.2 msec duration, 60 Hz). Respiratory rate, HR, PD, EEG and analgesia were monitored. Analgesia was assessed by measuring the latency of the skin twitch reflex (STRL) evoked by thermal stimulation.

ES produced mydriasis, a decrease in EEG, and analgesia. When the latter effect was observed there was concommitant urination and defecation. In some animals tachypnea, tachycardia and struggling or limb extension was seen. FEN produced a dose-dependent increase in EEG and a dose-dependent decrease in HR. FEN (9 µg) significantly increased the STRL and produced miosis. FEN had a calming effect and the fore- and hindlimbs became relaxed. NTX pretreatment antagonized FEN induced analgesia and bradycardia but not miosis or increased EEG. However, NTX alone produced significant miosis and increased EEG. MOR (57 µg) and FEN (9 µg) increased the STRL to approximately the same extent. However, MOR did not change PD, HR or EEG. Both EKC and nicotine failed to significantly alter any parameter studied.

These data demonstrate that the effects induced by microinjected FEN, except analgesia, are opposite to those produced by ES of the PAG. Further, i.v. NTX antagonizes some effects of FEN but not others. Lastly, FEN or MOR injection into and ES of the PAG produces analgesia. In this regard the dog is similar to the rat, cat and monkey.
(Supported by the University of Kentucky Tobacco and Health Research Institute.)

61.7 ENKEPHALIN SHORTENS Ca^{++}-SPIKES AT EARLY STAGES IN EMBRYONIC SPINAL NEURONS IN VIVO. John L. Bixby & Nicholas C. Spitzer, Biology Dept., University of Calif., San Diego, La Jolla, CA 92093

Rohon-Beard cells of the embryonic Xenopus spinal cord have proven useful for the study of vertebrate neuronal differentiation. Previous studies have demonstrated that these cells develop somatic Ca^{++}-dependent action potentials at the time of closure of the neural tube, and that the ionic dependence of these spikes shifts to Na^{+} over the next several days of development. Since immunocytochemical results suggest the presence of enkephalin in the dorsal spinal cord of Xenopus tadpoles (Lamborghini & Karten, pers. comm.), and since Ca^{++}-dependent action potentials of chick DRG cells in vitro are shortened by enkephalin (Mudge et al., PNAS 76: 526 (1979)), we examined the effect of enkephalin on the Ca^{++} spikes of Rohon-Beard cells. Our results indicate that met-enkephalin, which has no effect on the resting membrane voltage or conductance, reversibly and specifically shortens Ca^{++} spikes of Rohon-Beard cells in vivo.

Met-enkephalin, when applied to Rohon-Beard neurons by pressure ejection from a micropipette, reversibly reduces the duration of Ca^{++} spikes in concentrations as low as 1µM, and sometimes at 0.1 µM. Other opiate peptides (β-endorphin, dynorphin, leu-enkephalin) also reduce action potential duration at a concentration of 10 µM. In addition, 20 µM met-enkephalin shortened Ca^{++}spikes in Xenopus DRG neurons in vivo.

The Rohon-Beard cell enkephalin response is specific in that it is reversibly abolished by 1 µM naloxone. There is no apparent "desensitization" of the response for application times at least as long as 1 minute. This response to enkephalin could inprinciple be due either to effects on outward current(s), or to effects on Ca^{++} currents, but the following suggests that the latter is the case. 1)In mature cells, with a largely Na^{+}-dependent spike, there is no effect of enkephalin on spike height or duration; 2)enkephalin shortens Ca^{++}spikes of mature neurons that are elicited in high (∿20 mM) concentrations of TEA^{+}; 3)enkephalin reduces the overshoot of Ca^{++}spikes and can block the spike completely, suggesting that the effect is not on Ca^{++}-dependent K^{+} channels, which open with some latency upon spike initiation.

Examination of Rohon-Beard cells at various stages of development shows that enkephalin shortens Ca^{++}-dependent action potentials from the earliest times the spikes appear (∿21 hrs, the time of closure of the neural tube) to the latest times they are detectable (7-9 days, in the presence of TEA^{+}). This very early appearance of the response to enkephalin is consistent with the idea that the enkephalin binds directly to the Ca^{++}channels, or that an "enkephalin receptor" is among the earliest neuronal phenotypes to differentiate in these vertebrate neurons.
Supported by grants from the NIH and the ONR.

61.8 COMPARISON OF THE EFFECTS OF IONTOPHORETICALLY APPLIED INTERFERON AND MORPHINE ON THALMIC AND CORTICAL NEURONS. C. Reyes-Vazquez, B. Prieto-Gomez*and N. Dafny. Department of Neurobiology, School of Medicine, University of Texas, Houston 77025 and Departamento de Fisiologia, Facultad de Medicina, UNAM, Mexico.

A few studies have shown that interferon (IF) has an effect on excitable cells. Recent studies suggested structural and functional similarity between human leukocyte IF, ACTH and endorphins. The present study is undertaken to compare the effects induced by microiontophoretic application of IF with those elicited by morphine (MOR) in the cortex and medial thalamus. Male Sprague Dawley rats, anesthetized with urethane and mounted in a stereotaxic frame were used. An array of 5 micropipettes were used for microiontophoresis, containing: (1) recombinant leukocyte A IF (Hoffmann-La Roche), (2) MOR sulfate, (3) L-glutamate (GLUT), (4) naloxone hydrochloride (NAL) and (5) pontamine sky blue. The recording electrodes were filled with NaCl 4 M and glued alongside the multibarrel. The recording session for each cell included several segments of 260 sec. Spontaneous activity (60 sec) and the effects of 20, 50 or 100 nA of any of the drugs tested. The injection period for each drug was 60 sec and the electrical discharges were recorded for an additional 140 seconds post-drug effect for each dose. Eleven cortical and 9 medial thalmic units were tested for the effects of IF, MOR and GLUT. In all 11 cortical cells IF elicited a delayed and long lasting increase of spontaneous activity in dose response characteristics. In medial thalamus only 3 units were affected by IF in the same way. Higher currents of IF produce in the majority of the cells a decrease in the size of the action potential. NAL had different effects upon the changes induced by IF. In the cortical cells the application of GLUT and MOR produced as expected an increase and a decrease in a dose response manner respectively. However, in the thalamus 5 units were depressed by MOR, 2 were increased and 2 were not affected. Only the decreases induced by MOR in both structures were blocked by NAL. GLUT affected all 9 thalamic cells increasing their discharge. The present data show that the effects induced by microiontophoretic application of IF are different from those induced by MOR in the sites tested.

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61.9 OPIOID PEPTIDES SELECTIVE FOR MU- AND DELTA-OPIATE RECEPTORS DECREASE DURATION OF DRG NEURON CALCIUM-DEPENDENT ACTION POTENTIALS M.A. Werz and R.L. Macdonald. Neurosciences Program and Dept. of Neurology, The University of Michigan, Ann Arbor, MI 48109.

Opioid peptides decrease somatic calcium-dependent action potentials (CAPs) of DRG neurons grown in cell culture (Mudge et al., PNAS 70:526, (1979); Werz & Macdonald, Brain Res. 239:318 (1982)). The opioid peptide action is dose-dependent and naloxone reversible, suggesting that opiate receptors are present on the somatic membranes of DRG neurons in culture. Since multiple types of opiate receptors have been demonstrated in both peripheral and central nervous systems and since both mu- and delta-opiate receptors have been demonstrated on primary afferents, we have attempted to determine: 1) if they are present on DRG somata and 2) the correspondence of these receptor types to opiate mediated decreases of CAP duration. Using single DRG neurons we have investigated the potency of opioid peptides, leucine-enkephalin (L-ENK) and morphiceptin (MC), which have differential affinity for mu- and delta-opiate receptors. We predicted that L-ENK would be 1000 fold more potent than MC and the responses would not be highly naloxone sensitive if delta-receptors mediated opiate effects. The ligands would be approximately equipotent and the responses would be highly naloxone sensitive if mu-receptors mediated the opiate actions.

Cell culture and intracellular recording techniques were as previously described (Werz & Macdonald, Brain Res 239:318, 1982). Opioid peptides were applied by pressure ejection from micropipettes with tip diameters of 2-5 μm. NAL was applied by diffusion from micropipettes with tip diameter of 10-15 μm which were positioned 10-15 μm from DRG neurons.

The pattern of response of DRG neuron CAPs to L-ENK and MC was heterogeneous. L-ENK and MC decreased the duration of CAPs equipotently in a proportion of DRG neurons, consistent with mediation by mu-receptors. Two additional findings support mediation by mu-receptors. Firstly, large decreases in CAP duration were produced by 1 μM MC, a concentration more than one log unit below the half maximal effective dose in the delta-receptor predominant mouse vas deferens. Secondly, DRG neurons which responded equipotently to MC and L-ENK were highly sensitive to NAL antagonism. In contrast, a proportion of DRG neurons were highly sensitive to L-ENK, did not respond to MC and required high concentrations of NAL for antagonism of L-ENK responses, consistent with the opiate effects being mediated by delta-receptors. Therefore, our results suggest that both mu- and delta-receptors are present on the cell bodies of DRG neurons in cell culture and that both receptor types mediate decreases in somatic CAPs of DRG neurons.

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61.10 RELEASE OF ENKEPHALIN WITHIN THE HIPPOCAMPUS - INABILITY TO DEMONSTRATE USING NALOXONE BLOCKADE. M.A. Linseman and W.A. Corrigall. Neurobiology Section, Addiction Research Foundation, Toronto, Ontario, Canada, M5S 2S1.

Direct application of enkephalin has been shown to augment field potentials and unit activity of hippocampal pyramidal cells and to result in epileptiform activity in the EEG. In addition, various investigators have demonstrated both opiate binding sites and endogenous opiate ligands within the hippocampus. These data suggest that enkephalins may be released under certain circumstances within the hippocampus as a neurotransmitter or neuromodulator. In order to determine how enkephalins may be released within the hippocampus and, possibly therefore, what function they might mediate, we have carried out two experiments.

In the first experiment we examined the effect of 2 mg/kg naloxone i.v. in a series of acute and chronic electrophysiological experiments. These experiments examined naloxone effects on:

1) singly evoked monosynaptic field potentials of CA1, CA3 and the dentate gyrus,
2) CA1 responses that were enhanced by a particular pattern of stimulation, as in potentiation,
3) hippocampal responses that were augmented by prior extra hippocampal stimulation of the medial or lateral septum (Krnjevic and Ropert, Can. J. Physiol. Pharmacol. 59: 911: 1981), midbrain reticular formulation (Bloch and Laroche, Behav. Brain Res. 3: 23:1981), or raphe nuclei during slow wave sleep (Winson, J. Neurophysiol. 44: 937:1980).

In all cases naloxone was without effect.

In a second behavioral experiment, we have used the 8-arm radial maze to examine the effect of naloxone on supramaximal stimulation of the perforant path during a delay period interposed between the first four and the last four choices. We found no amelioration by naloxone (5 mg/kg s.c.) of the performance deficits produced by stimulation (nor any effect of naloxone on maze performance in the absence of stimulation). This is consistent with the observations from our recording experiments, but is in contrast to the positive results reported for naloxone in blocking the disruption of a similar hippocampal spatial memory task due to stimulation of the dentate granule cells (Collier et al. Soc. Neurosci. Abstr. 7: 359:1981).

We conclude that enkephalins are not released by electrical stimulation of any of these pathways with the parameters we have used (but may be by other as yet unknown or untested pathways), that the presence of some cofactor may be necessary for release of enkephalins by such stimulation, or that enkephalins are released under conditions other than electrical stimulation of the brain.

(Supported by the Addiction Research Foundation of Ontario.)

61.11 MORPHINE PRODUCES TIME-DEPENDENT CHANGES IN LOCOMOTOR ACTIVITY AND IN DISCHARGE RATES OF MESENCEPHALIC DOPAMINE NEURONS. N.L. Ostrowski, I.A. Paul*, M. Drnach* and A.R. Caggiula. Psychobiology Program, Psychology Department, University of Pittsburgh, Pittsburgh, PA 15260.

Intravenous (IV) administration of a very low dose of morphine sulfate (10 ug/kg), but not dextrorphan, increased the extracellularly recorded firing rates of Type A DA cells (neurons activated by tail pinch) and decreased the firing of Type B DA cells (inhibited by tail pinch; Chiodo et al., 1980) in the substantia nigra-ventral tegmental area (SNC-VTA) of chloral-hydrate anesthetized male rats. Type A neurons showed a gradual increase which lasted about 15-20 min. Type B cells, however, showed little change initially, but demonstrated a marked suppression of firing beginning between 6 and 9 min following morphine, and lasting 30-60 min in some neurons. Since pre- or post-treatment with 0.1 mg/kg of naloxone antagonized the increases in Type A cells while only pre-treatment with 5.0 mg/kg of naloxone blocked morphine's effect on Type B cells, both responses probably involve opioid receptors, although the underlying mechanisms of action may differ.

Behaviorally, the same dose of morphine that altered discharge rates of DA neurons produced time-dependent changes in locomotor activity of awake, male rats. Animals that had been habituated to an open field before and after jugular catheterization, responded to 10 ug/kg (IV) of morphine with an increased amount of activity initially, and suppressed activity by 30 min relative to control rats (drug x time interaction, $p < .02$). Saline-treated rats (n=8) entered 156, 69 and 59 blocks in an open field during three consecutive 10-min tests, while morphine-treated rats (n=8) entered 218, 72, and 32 blocks. Similarly, morphine-treated rats spent a greater proportion of time engaged in forward locomotion during the first ten min after drug, and a lesser proportion of time during the third ten min after drug, than saline-controls (drug x time interaction, $p < .02$).

These results show that the same low dose of morphine produces parallel changes in the activity of mesencephalic DA cells and in a behavior previously linked to DA function. Future attempts to determine the nature of this relationship will rely on the correspondence or lack of correspondence between these electrophysiological and behavioral effects with respect to naloxone reversibility, time course and dose-response patterns.

61.12 CALCIUM DEPENDENT POTASSIUM CONDUCTANCE IS ENHANCED AFTER PROLONGED MORPHINE TREATMENT. T. Tokimasa,* E. Cherubini* and R. A. North. (SPON: M. Fernstrom). Neuropharmacology Laboratory, Department of Nutrition and Food Science, M.I.T., Cambridge, MA 02139.

Intracellular recordings were made from neurons in myenteric plexus of the guinea-pig ileum. Guinea-pigs were either naive or pretreated with multiple implantations of morphine pellets over several days. From either group of animals, tissue was placed in normal Krebs solution or in solution containing morphine (30 nM - 1 μM). A calcium-dependent potassium conductance was shown to be responsible for the hyperpolarization which followed either a single or a train of action potential(s). A similar hyperpolarization followed a nicotinic depolarization evoked by iontophoresis of acetylcholine (in presence of atropine 100 nM) to the soma membrane of cells that showed a fast e.p.s.p. Cells never exposed to morphine (in vitro or in vivo) showed small or absent calcium dependent potassium conductances. Cells exposed to morphine for many hours (in vitro or in vivo) showed large calcium-dependent potassium conductances. This effect of chronic morphine exposure was qualitatively similar to the acute action of morphine on naive neurons (Tokimasa et al. Nature 294:162, 1981), but much more marked in degree. The progressive development of this change during long term exposure to morphine may result from a changing ability of an intracellular calcium control mechanism, and may be causal in the cellular mechanism of morphine dependence.

62.1 SHIVERER MICE: ULTRASTRUCTURAL, ORGANOTYPIC CULTURE, AND IMMUNOCYTOCHEMICAL OBSERVATIONS ON CNS. S. Billings-Gagliardi, R. L. Sidman and M. K. Wolf. Department of Anatomy, University of Mass. Medical School, Worcester, MA 01605, and Department of Neuropathology, Harvard Medical School, Boston, MA 02115.

The shiverer (shi) mouse mutation impairs CNS myelination: myelin is grossly reduced in amount, lacks myelin basic proteins (MBP), and usually fails to form the major dense line. The shi mutation is being transferred to a B6C3H hybrid stock congenic with other CNS hypomyelinated mutants by standard cross-intercross matings. Affected animals and their controls at N3 (87.5% congenicity) have been studied intact and in organotypic culture by light and electron microscopy, and by MBP immunocytochemistry on 1µm Epon sections according to the peroxidase-antiperoxidase method (Trapp, B. D., et al., J. Cell Biol. 90: 1, 1981) using goat anti-rabbit MBP given to RLS by Cohen and Sternberger. On the new background, shi/shi animals have more CNS myelin than previous shi stocks at P-21 to P-23, but the myelin is still non-compacted and MBP-. While some myelin sheaths are formed by spiraled processes, the majority are formed by stacks of cytoplasmic sheets which may surround only part of an axon's circumference. In addition, numerous slender oligodendrocyte processes without apparent axonal association may be gathered together in sheaves. Affected animals are intercrossed to obtain 100% shi/shi litters for organotypic culture of P-0 cerebellum. At 20-22 days in vitro, cultures are normal except for myelin, which replicates the major ultrastructural features of the disease and is MBP-.

Myelination in organotypic cerebellar cultures from other hypomyelinated mutants (jp, jp^{msd}, qk) is significantly increased by co-culture with normal optic nerve (Billings-Gagliardi, S., et al., Neurosci. 7: 911, 1981). It has been questioned whether the additional myelin is directly produced by the introduced normal oligodendrocytes. In cultures of shi/shi cerebellum with added optic nerve from normal P-8 to P-10 B6C3H mice, numerous MBP+ myelin segments are present in the cerebellar explant close to the optic nerve explant. Other areas of the same cerebellar explant show MBP- myelin. TEM study of these cultures shows "normal" and "shi" types of myelin ultrastructure in a similar distribution. The production of two distinct types of myelin by the same culture strongly supports the idea that the MBP+ myelin is formed by normal optic nerve oligodendrocytes directly myelinating mutant axons, and suggests that oligodendrocytes are primarily responsible for the myelin abnormality in the shiverer mouse.

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62.2 MYELINATION OF CYTOSINE ARABINOSIDE TREATED CEREBELLUM CULTURES BY OLIGODENDROCYTES FROM NORMAL OPTIC NERVE. Gail Schwing-Stanhope* and Merrill K. Wolf. Department of Anatomy, University of Massachusetts Medical Center, Worcester, MA 01605.

Myelination in organotypic cerebellar cultures from hypomyelinated mutant mice (qk, jp, jp^{msd}) is significantly increased by co-culture with normal optic nerve. Presumably, the mutant axons accept myelination by normal oligodendrocytes. We sought a means to do the complementary experiment: to confront normal axons with mutant oligodendrocytes. Seil and Blank (Science 212:1407, 1981) reported that cytosine arabinoside (Ara-C) prevented myelination in cultures of normal cerebellum, by suppressing oligodendrocyte differentiation. Post-mitotic neurons were spared and myelin could be restored by adding another cerebellar explant in which neurons had been chemically destroyed. We now confirm that Ara-C can prevent myelination in normal cerebellar explants, and find that myelin can be restored by adding normal optic nerve after ceasing Ara-C treatment.

Cultures were prepared from newborn unaffected littermates of our B6C3H hybrid jp and jp^{msd} stocks. Two cultures from each cerebellum were treated with Ara-C (5 µg/ml in medium without embryo extract) for 7 days in vitro (DIV) while the other two received the same medium without Ara-C. After 7 DIV, the cultures were washed and fed medium with embryo extract but without Ara-C. One of each pair of Ara-C treated cultures received an additional explant of optic nerve from a 7 or 8 day old normal mouse. At 16 DIV all untreated cultures showed abundant visible myelin. Ara-C treated cultures without optic nerve showed no myelin. This was confirmed by aldehyde/osmium fixation, Epon embedment and examination of semithin sections. Large neurons survived, even in regions of destruction of other tissue elements. 75% of Ara-C treated cultures with optic nerve showed areas containing heavily myelinated axons radiating from the optic nerve explant.

Our cultures required 7 days of Ara-C treatment rather than 5 as reported by Seil and Blank. Lower dose, shorter time, or embryo extract in the medium all prevented a complete suppression of myelination. In our cultures, unlike those of Seil and Blank, granule cells were reduced in numbers but not totally absent. Some granule cells are probably postmitotic at birth in our mice.

These experiments show that Ara-C treatment produces cultures which contain intact axons receptive to myelination but lack competent oligodendrocytes. Such cultures should be a suitable system in which to test the capacities of oligodendrocytes from mutant optic nerve.

Supported by NIH grant NS 11425.

62.3 A MODEL FOR STUDYING A POTENTIAL NEGATIVE FEEDBACK MECHANISM ON THE PROLIFERATION OF NEUROEPITHELIAL CELLS IN CULTURE. J. Houle and S. Fedoroff. Dept. of Anatomy, Univ. Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 0W0.

Previous studies of spinal cord neurogenesis in vivo (Nornes and Carry, 1978) have indicated a strict adherence of neuroblast formation to an orderly spatial and temporal sequence. In an attempt to establish the possible role played by humoral factors on this patterned cell production we have developed an in vitro model to examine the proliferation, migration, and differentiation of neuroblasts from fragments of embryonic mouse neural tube. We were particularly interested in studying the response of this pluripotent cell population to possible trophic factors naturally present in embryos of later gestational stages.

A four somite length of brachial neural tube was dissected from 10-day-old (30-34 somites) mouse embryos and cut into 32 fragments equal in size though not necessarily equal in cell composition. Fragments were placed on collagen coated coverslips in a modified Eagle's MEM with 5% horse serum and 0.8 µg/ml insulin. Extracts from either whole embryos or brain prepared from mice of various gestational and postnatal stages were added for the initial 10 days of culturing at various concentrations. Fragments cultured without extracts served as controls. After 21 days in culture neural tube fragments were fixed and stained with thionin, protargol silver, or for acetylcholinesterase. The outgrowth zone of individual fragments was examined with a Zeiss image analyzer for extent of cell migration, cell size distribution and estimated number of neurons formed from each fragment.

Fragments in control cultures were of 2 different appearances. Those that were totally flattened extended from 1-3 mm^2 in area and were comprised primarily of neurons 14-24 µm in diameter atop a glial layer. The mean number of neurons per mm^2 was estimated to be 208 ± 16. The majority of fragments however consisted of a central dense core composed of large-medium sized neurons surrounded by an outgrowth zone of neurons on a glial layer. The outgrowth area measured from 3-10 mm^2 with most neurons in a size range of 8-14 µm in diameter. The number of neurons per mm^2 was estimated to be 1200 ± 206. The effects of embryo extract present during cell proliferation was related to the concentration employed and the age of tissue used. In general there appeared to be an inhibitory action on neuronal precursor cell proliferation as determined by a decrease in the outgrowth zone and neuron density. Quantitative evidence will be presented demonstrating a greater reduction in cell number (i.e. less proliferation) when extracts from later stage embryos were used. (Supported by Grant MT4235 from Medical Research Council of Canada.)

62.4 SERUM-FREE CULTURE CONDITIONS FOR DISSOCIATED POSTNATAL MOUSE CEREBELLAR CELLS: A CONTROLLED STUDY OF CELL ATTACHMENT AND SOME CULTURE PARAMETERS, S.Huck and H.Ditrich*. Inst. of Neuropharmacology, University of Vienna, A-1090 Vienna, Austria.

Serum-free culture conditions offer several advantages over serum-supplemented media. This contribution deals with two aspects arising from the deletion of serum: improved attachment of cerebellar cells to the substratum, and persistence of variables affecting culture survival and appearence in spite of the serum free conditions. Results are based on our recent observation that dissociated postnatal mouse cerebellar cells can be maintained serum-free in basal medium Eagle (BME) supplemented only by insulin (special FEBS meeting, April 1982, Athens).

Dissociated mouse cerebellar cells (from trypsinized and triturated cerebella of 7 day old animals) readily adhered to tissue culture dishes (Nunc) if seeded in insulin-supplemented BME (adhesion beeing defined as the ratio of attached to floating cells which were counted 15 and 40 minutes after plating). In contrast, attachment was greatly reduced if cells were seeded in serum supplemented medium. Likewise, pretreatment of tissue culture dishes with serum or bovine serum albumin deminished attachment of the cells. However, if dishes were coated with poly-D-lysine, the attachment was as good for cells resuspended in serum-supplemented medium as for those resuspended serum-free (in the latter case attachment was unchanged by the polyamine coat). The results were similar if plastic petri dishes were used instead of the tissue culture material, except that poly-D-lysine of course could not improve adhesion of cells in the presence of serum. Without serum, cells were also found to adhere well to untreated, gold plated and Si_3N_4-coated (an electrical insulator) cover glasses.

Although a better control of variables is guaranteed by serum free media (SFM), culture conditions cannot yet be regarded as completely defined. First, even basic media like BME contain unknown amounts of impurities. This may explain our observation that several batches of BME yielded greatly differing results: from no neurons surviving a 1 week incubation up to a survival rate of 40-60%. It is interesting to note that cultures were remarkably resistent to pH and osmotic deviations of the culture medium. Second, we observed differences in the appearence of cultures suggestive of "self-conditioning": if culture medium was renewed 1 day after plating, the cells remained disperse in a monolayer. In contrast, unfed cultures arranged themselves in small reaggregates by the third day in vitro.

To sum up, this contribution recalls and extends a previous observation, that SFM may increase the adhesion of neurons to the substratum (Ludueña, 1973), making serum-free neuronal cultures more generally applicable. More work will be required to completely define culture conditions, even in the absence of serum.

62.5 THE GROWTH AND SURVIVAL OF DISSOCIATED CELL CULTURES OF THE CNS IN SERUM-FREE, CHEMICALLY DEFINED MEDIUM. Douglas A. Kniss and Richard W. Burry, Department of Anatomy, College of Medicine, The Ohio State University, Columbus, Ohio 43210

The growth and survival of dissociated cell cultures of the central nervous system was examined in chemically defined, hormone supplemented medium. Dispersed cell cultures of 2-day old rat cerebellum were exposed from 9 to 21 days in vitro to chemically defined medium which consisted of Ham's F-12 plus the following constituents: putrescine (100μM), selenium (30nM), insulin (5μg/ml), and transferrin (100μg/ml). In addition, this medium was supplemented with one of the following compounds: progesterone (20nM), corticosterone (.02-200μM), hydrocortisone (.02-200μM), fibronectin (2.5-10μg/ml), or mixed gangliosides (5-400μg/ml).

Phase contrast microscopy and Trypan Blue exclusion showed that serum-free medium supplemented with .02-200μM corticosterone or hydrocortisone was more effective in promoting survival of neurons and non-neuronal cells than was progesterone at levels of .02-200μM. In addition, non-neuronal cells in both progesterone cultures and in cultures without any hormone supplements tended to be rounded up and retracted away from the culture dish. In contrast, in corticosterone and hydrocortisone treated cultures non-neuronal cells tended to maintain a flat sheetlike morphology for a longer time.

Fibronectin was also effective in promoting longer survival of cultures than serum-free medium without any supplementation. Non-neuronal cells tended to maintain their monolayer character in fibronectin-containing cultures. Mixed gangliosides at levels of 5-400μg/ml were completely ineffective in maintaining growth and survival when added to serum-free medium. In fact, with concentrations as low as 50μg/ml nearly all neurons and non-neuronal cells were dead within 48 hours after initial exposure.

These results suggest that supplementation of serum-free, chemically defined medium with glucocorticoid hormones or fibronectin but not progesterone or mixed gangliosides can have growth and survival promoting effects in cerebellar dispersed cell cultures. Supported by NIH Grant NS-15894.

62.6 CHARACTERIZATION OF Y79 HUMAN RETINOBLASTOMA CELLS AS AN IN VITRO RETINAL MODEL. B.T. Hyman, M.A. Yorek*, D. Dudley* and A.A. Spector*, Department of Biochemistry, Univ. of Iowa, Iowa City, Iowa 52242.

Clonal neural cell lines exhibit significant experimental advantages over primary explant cultures in that they provide an homogenous source of material in a rapidly growing system. In an attempt to develop a retinal model, we have investigated some characteristics of the Y79 human retinoblastoma cell line. Because retina is embryologically identified as central nervous system, this cell line may be a useful model for the central nervous system. The retinoblastoma cells grow in suspension culture in RPMI 1640 medium supplemented with 10% fetal bovine serum and have a doubling time of 81 h. In addition, the cells can be maintained in a serum-free culture medium. The retinoblastoma cells have both high- and low-affinity uptake systems for choline and glycine. The high-affinity systems exhibit a K_m' of 2.16 ± 0.11 and 34.2 ± 3.7 μmoles and a V_{max}' of 27.0 ± 2.9 and 91.2 ± 16.2 pmol min^{-1} mg $protein^{-1}$ for choline and glycine, respectively. High-affinity choline uptake is Na^+-independent, whereas the high-affinity uptake of glycine is Na^+-dependent. In a study of polyunsaturated fatty acid metabolism, it was determined that the Y79 retinoblastoma cells have the ability to synthesize docosahexaenoic acid (22:6 n-3) from linolenic acid (18:3 n-3), the precursor of the n-3 class of polyunsaturates. High contents of 22:6 n-3 are characteristic of the retina and central nervous system; no other cultured cell line has been reported to have this metabolic capability. When incubated in a serum-free medium, radioactive 22:6 is incorporated preferentially into the ethanolamine glycerophospholipid fraction. This is consistent with the observation that the ethanolamine glycerophospholipid fraction of neural tissue in vivo is enriched in 22:6. The membrane resting potential of Y79 retinoblastoma cells is -58.0 ± 0.7 mV for cells in a buffer containing 5 mM K^+. When K^+ was substituted isomotically for Na^+, the resting potential decreased; above 50 mM K^+, the slope of decrease was -59 mV/decade (a "Nernst" slope), suggesting that the membrane was acting as a permselective barrier for K^+. Depolarizing retinoblastoma cells with 50 mM K^+ stimulates the cells to rapidly and specifically release glycine, a putative neurotransmitter in the retina and parts of the central nervous system. Maximal glycine release occurs after 1 min and is 2 to 3 times greater than basal release measured either by radioisotope or amino acid analysis. These results agree closely with that reported in the literature for other model systems and suggest that the Y79 retinoblastoma cells are of neural origin, making this line a potentially useful in vitro model of the neuroretina. (Supp. by NIH grants HL 14230, CA 09118 and GMO 7337).

62.7 NEURITE OUTGROWTH FROM FETAL RAT HYPOTHALAMIC NEURONS ON DIFFERENT SUBSTRATES. K. A. Elias*, P. C. Goldsmith, and R. I. Weiner. Department of OB/GYN & Reproductive Sciences, University of California, San Francisco, California 94143.

The outgrowth of neurites from dispersed hypothalamic neurons cultured on plastic is delayed for several days until glial elements have proliferated to form a basal layer. In the present study, we have compared the efficacy of culture dishes alone or coated with polylysine, rat tail collagen matrix or extracellular matrix to promote neurite outgrowth. Rat tail collagen was diluted with acetic acid and polymerized with a NaOH : Waymouth media mix to form a matrix. Extracellular matrix was produced from bovine corneal endothelial cells by the method of Gospodarowicz (J. Biol. Chem., 253:3796, 1978). Hypothalamic and preoptic/septal areas were dissected from 18-day gestational age rat fetuses. The fragments were mechanically dispersed and plated on the various substrates at a density of 8 X 10^5 cells per 35 mm well. Cells were cultured in Dulbecco's Modified Eagle's medium containing 1% penicillin-streptomycin. 0.1% gentamycin, 1% fungizone, 10% fetal calf serum, and 5% calf serum. Neurons were identified by: phase contrast microscopy by their phase bright appearance and presence of growth cones; positive immunohistochemical staining with antibodies against neurofilaments (Eng A15K absorbed with glial fibrillary acidic protein (GFA)) and negative staining with an antibody to GFA (Eng R15 G+H). Cells were fixed with paraformaldehyde and immunohistochemically stained by the peroxidase-antiperoxidase technique. No significant neurite outgrowth was observed from cells on plastic until 72h. Neurite outgrowth on polylysine and extracellular matrix were similar (36 ± 2 and 47 ± 7 μm/day for the first 24h) and significantly higher than on plastic at all times. However, neurite extension was most rapid on the rat tail collagen matrix (70 ± 7 μm/day for the first 24h). After 72h, neurite outgrowth from cells plated on rat tail collagen was 2 times greater than those plated on extracellular matrix or polylysine and 14 times greater than cells grown on plastic. The mechanism by which polylysine augments neurite outgrowth appears to involve the potentiation of the attachment of neurons to the culture dish by electrophilic bonding. The mechanisms by which rat tail collagen and to a lesser degree extracellular matrix promote neurite extension is unclear, but is likely to involve both cellular adhesion as well as the presence of growth promoting factors.

This work was supported by NIH Grant HD-08924 and The Mellon Foundation.

62.8 ULTRASTRUCTURE OF DOG STRIATAL NEURONS IN ORGAN CULTURE. Catherine Mytilineou, Mount Sinai School of Medicine, New York, N.Y. 10029

Striatal tissue from newborn mongrel dogs was set in organ culture alone or in co-culture with substantia nigra in the Maximow chamber double coverslip assembly. After 7, 13, 27, 45-54 and 80-95 days in vitro (DIV) selected cultures were fixed in 2% gluteraldehyde and processed for electron microscopy. Some cultures were incubated with 10^{-3}M 5-hydroxydopamine (5-OHDA) at 37°C for 10 min. before fixation. After 7 DIV the neurons appear very immature with only a thin rim of cytoplasm containing few organelles around an ovoid nucleus. Immature processes with accumulations of large vesicles near the plasma membrane and growth cones occupy spaces between cellular debris. Neuronal types cannot be identified at this stage of development. At 13 DIV most of the neurons are still immature resembling bipolar neuroblasts. Neurons at intermediate stages of development and mature looking neurons are also present. The size (more than 20u) and the morphological characteristics of the more mature neurons (deeply indented nucleus, pale mitochondria, complex Golgi cysternae) resemble the large size neurons described in the adult neostriatum in situ. Growing processes and immature synaptic junctions are filling the intercellular spaces. At 27 DIV both neurons and their dendritic and axonal processes appear mature. However, the size of the neurons continues to increase for the following two weeks. Several growing neuronal processes are also present at this stage of development. Three types of neurons are present in the mature cultures of striatum: small size (less than 10u), medium size (12-20u) and large size neurons (more than 22u diameter). The medium size neurons which are the most numerous can be subdivided into 3 groups according to their ultrastructural characteristics. (1) Neurons with smooth round nuclei and with no apparent Golgi apparatus in the cytoplasm around the nucleus; (2) neurons with slightly elongated nucleus with small invaginations and cytoplasm rich in Golgi and lysosomes; (3) neurons with deeply indented nucleus, many free ribosomes forming rosettes and accumulations of rough endoplasmic reticulum. The most common synaptic profiles in the striatum grown alone are en passant making assymetric contacts with medium sized dendrites. Boutons making synaptic contacts with dendritic profiles, axosomatic and few axospinous synapses are also present. When substantia nigra is co-cultured with the striatum the number and types of axonal boutons present in the striatum increases significantly. The dopaminergic boutons labelled with 5-OHDA make assymetric synaptic contacts with small dendritic branches or dendritic spines Supported by N.I.H. Grant NS-11631.

62.9 ULTRASTRUCTURAL ANALYSIS OF DISSOCIATED CILIARY GANGLION CELLS CULTURED UNDER DIFFERENT CONDITIONS, G. Crean, Physiology Section, The Biological Sciences Group, Univ. of Conn, Storrs, CT 06268

When ciliary ganglion cells are dissociated and cultured in the presence of muscle, conditioned media, or embryo extract they survive and grow in long term culture systems. Since growth in culture is dependent upon survival, using ultrastructural techniques we studied the neurons, both living and dying cells and during their growth under various culture conditions to define what role the culture environment plays in the fine structural organization of the cell soma and its processes.

Ciliary ganglion cells were cultured in growth media containing MEM, additional a.a.'s, vitamins, horse serum and chick embryo extract. Sister cultures were grown in growth media minus chick embryo extract. Growth rate and ultrastructural morphology was studied over a three week period. Results show that at the time of plating cell diameters ranged from 10-12 um. After 24 hrs. in growth media with CEE cell diameters averaged 13-14 um while neurons in extract deficient media began to demonstrate aggregative properties with 4 to 6 cells in a cluster. As early as 4 hours after plating neurons not exposed to CEE at dissociation demonstrate an electron dense cytoplasm, electron light nuclear envelope, elongated mitochondria, and few processes and growth cones while neurons plated in media containing extract showed a distinct nuclear envelope, evenly patterned nuclear pores, neurite outgrowths and growth cones. Within 24 hrs neurons in extract deficient media are grouped into clusters. These clusters consist of flattened neurons with few or no processes and non-neuronal cells full of lysozomal granules. In contrast, neurons plated with extract have well developed processes with a round morphology. Immature synapses can be readily seen and a few more highly developed small synapses are also present. At about 5-6 da in culture neurons grown in extract deficient media have virtually all died and the cells in extract supplemented media have increased in size to almost 20 um. Ultrastructurally this rapid growth is attributed to a marked increase in the number of ribosomes which are initially free and then become attached to the endoplasmic reticulum. The rest of the soma undergoes reorganization of somal mitochondria, ribosomal material and neural filaments. This ultrastructural analysis has shown that the culture environment does influence the time and degree of organization of organelles in the neuronal cell soma and its processes.
Supported by NIH MS 10338 and the U.S. Army Research Office.

62.10 MOUSE SPINAL CORD IN DISSOCIATED CELL CULTURE - SEPARATE CULTURE OF DORSAL AND VENTRAL HALVES. Peter B. Guthrie and Douglas E. Brenneman. Lab Developmental Neurobiology, National Institute of Child Health and Human Development, NIH, Bethesda, MD., 20205.

The dissociated mouse spinal cord (SC) culture is a valuable model system for developmental and cellular neurobiology. One major problem with dissociated central nervous system cultures in general is heterogeneity. Does this heterogeneity reflect morphologically and functionally distinct sub-populations in the cultures?

To characterize sub-populations in SC cultures, we have attempted to reduce heterogeneity by culturing the dorsal-half (DH) and ventral-half (VH) of the SC separately. Embryonic mouse SC (13d.) were pinned dorsal side up onto SYLGARD (Dow-Corning) dishes and stripped of dorsal root ganglia and meninges. The cord could then be opened along the central canal with the lateral edges representing the dorsal SC and the inner section the ventral SC.

Cultures from the two halves showed consistant differences. The DH would only survive on pre-plated layers of non-neuronal cells and required supplemented medium (5% horse serum/MEM with added insulin, transferrin, selenium, putresine, corticosterone, progesterone and tri-iodothyronine). The DH cultures consisted primarily of small neurons that aggregated in spherical clusters.

The ventral-half survived well on collagen in normal growth medium (10% horse serum/MEM). The VH cultures consisted primarily of large neurons that remained well separated from each other. The larger size of the VH neurons was reflected by the 2-fold greater binding of ^{125}I-tetanus toxin. In addition the VH cultures contained greater than 75% of the choline acetyltransferase activity.

Each of these separate cultures resembles a portion of the standard dissociated mouse spinal cord culture. These results suggest that consistant morphological differences are maintained in the cultures, and that some of the heterogeneity of the spinal cord culture reflects heterogeneity within the in vivo spinal cord. We are now attempting to distinguish sub-populations within the separate DH and VH cultures according to morphological, biochemical and immunohistochemical properties.

62.11 PRODUCTION OF IMMUNOREACTIVE SUBSTANCE P (i-SP) BY RAT SENSORY NEURONS IN CULTURE AND THE EFFECT OF HERPES SIMPLEX VIRUS (HSV) ON i-SP CONTENT, R.J. Ziegler, R.S. Pozos, V. Seybold and M.M. Tuchscherer*, Depts of Med. Microbiology and Physiology, Univ. of Minn., Duluth and Dept of Anatomy, Univ. of Minn-Twin Cities, Duluth, Minn. 55812.

Cultures of dissociated, rat sensory neurons were prepared from dorsal root ganglia of 17-day rat fetuses. By using the procedure described by Wood (Wood, Brain. Res. 115:361, 1976) relative pure cultures of isolated rat sensory neurons were obtained. The soma of some neurons contained detectible immunoreactive-Substance P (i-SP) after three days of culture. By 14 days of culture about 40 % of the neuronal somas contained i-SP. No i-SP was observed in neuritic extensions.

When co-cultures of isolated spinal cord and dorsal root ganglia neurons were prepared and cultured by the same method, essentially the same scheme of i-SP detection was observed except that at 14 days of culture, i-SP was also observed in neuritic extensions. By employing a chambered system within the culture dish (Ziegler and Herman, Inf. Immun. 23:620, 1980. with spinal cord neurons and dorsal root ganglia neurons on opposite sides, we demonstrated that i-SP could only be detected in dorsal root ganglia sensory neurons or their neuritic extensions and that i-SP could be induced to appear in sensory neuronal extensions after treatment with spinal cord neurons conditioned media.

Infection of co-cultures with Herpes simplex virus (MacIntyre strain) at a m.o.i. of 50-100 indicated that i-SP was still present in infected neuronal somas and neuritic extensions at 24-30 hours post-infection. This observation is curious since HSV normally shuts down host cell protein synthesis shortly after infection and the turnover time of i-SP in rat dorsal root ganglia in vitro is 3.6 hours. (Harmar and Keen, Brain Res. 231:379, 1982).

62.12 DIFFERENTIATION MARKERS IN RAT SENSORY NEURONS IN CULTURE. P G Hogan* and P.I.Baccaglini (SPON: E. Furshpan). Neurobiology, Harvard Medical School, Boston, MA 02115

Several functional classes of sensory neurons are present in adult mammals. They differ in their connections in the periphery and the central nervous system, in their membrane properties, and in their histochemical staining properties. It would be useful for developmental studies if functionally distinct classes of sensory neurons could be recognized in cell culture.

We have examined rat sensory neurons, grown in culture in the absence of other cells, for expression of some physiological and histochemical properties of sensory cells.

Capsaicin sensitivity and menthol sensitivity are markers for restricted classes of sensory fibers in adult mammals. Capsaicin selectively excites unmyelinated pain sensory fibers, while menthol selectively excites cold sensory fibers.

We have found that both capsaicin and menthol excite sensory neurons in culture. Capsaicin elicited action potentials at concentrations as low as 1nM, and menthol at concentrations as low as 10μM. When we tested these compounds at a range of concentrations, to ensure that all sensitive cells were identified, capsaicin excited 70%-90% of the neurons. Menthol excited 10%-20%. These proportions may reflect the fact that there are more unmyelinated pain fibers than cold fibers in nerves of adult rats.

Neurons in culture which expressed capsaicin sensitivity usually had other properties in common with unmyelinated pain fibers. Among the capsaicin-sensitive cells, 94% were excited by low concentrations of bradykinin, 80% were excited or sensitized by low concentrations of prostaglandin E_2, and 66% were specifically stained by an antiserum to substance P.

Many cells were excited only by capsaicin, and some cells only by menthol, but an appreciable number were excited by both compounds. Capsaicin and menthol are reported to excite different classes of sensory fibers in adult mammals, but it may be that during development some cells are sensitive to both compounds.

Staining by antisera to certain peptides may also indicate different functional classes of sensory neurons. We have found that substance P, somatostatin, and vasoactive intestinal polypeptide, or related antigens, are present in the cultures. Relatively large numbers of neurons stain with antiserum to substance P, and relatively few with antiserum to vasoactive intestinal polypeptide, as in sensory ganglia from adult rats.

We conclude that markers for unmyelinated pain sensory fibers and for cold sensory fibers are expressed by sensory neurons in culture. Other histochemical markers, which might indicate the presence of other functional classes of cells, are also expressed.
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62.13 SURVIVAL REQUIREMENTS OF GANGLIONIC NEURONS IN SERUM-FREE CULTURES. Ivan Selak*, Stephen D. Skaper and Silvio Varon. Dept. Biol., Sch. of Med., Univ. Calif. San Diego, La Jolla, CA 92093.

Cultivation of neural cells in traditional media routinely requires serum supplementation and, sometimes, trophic factors. Simplification of medium composition to identifiable elements eliminates the complex and potential inhibitory influences from it. We have recently demonstrated that chick embryonic day 8 (E8) dorsal root ganglionic (DRG) neurons and E11 sympathetic ganglionic (SG) neurons, as well as various central neurons can be maintained in a serum-free medium using the N1 supplement, consisting of insulin, transferrin, putrescine, progesterone and selenite.

In the present report we have extended our studies to include a wider range of embryonic ages, species, and culture conditions. Chick SG neurons throughout the E8-E11 time span can be cultured with only insulin plus selenite, with added Nerve Growth Factor (NGF). An additional requirement for transferrin appears from E11 onward. Chick DRG neurons over a broad developmental period (E7-E15), as well as neurons from neonatal mouse DRG can be cultured in the absence of serum provided only insulin and transferrin are added to the medium, in addition to NGF. A ganglionic neuronotrophic factor (GNTF), derived from selected intraocular tissues of E15 chick, supports the same neuronal population from E11 chick SG and neonatal mouse DRG which NGF supports. Chick dorsal root ganglia from E10 onward also contain a population of neurons responsive to the eye-derived GNTF, however, which is distinct from the NGF-dependent subset. E10 and E15 chick DRG neurons supported by the eye-derived GNTF in the absence of serum require the additional supplementation of only insulin plus transferrin, as do their NGF-sensitive ganglionic counterparts. Neonatal mouse DRG neurons, whether supported by NGF or the eye-derived GNTF, also survive in serum-free medium with only insulin and transferrin supplementation. Experiments are in progress to define specific metabolic behaviors in DRG and SG neurons, which are affected by the required N1 constituents.

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62.14 IN VITRO VIRUS-ASTROCYTE INTERACTIONS WITH CYTOLYTIC AND NON-CYTOLYTIC NEUROTROPIC VIRUSES. B.R.Brooks*, R.L.Hirsch*, J. Gossage*, R.T.Johnson. Dept. Neurology and Microbiology, Univ. Wisconsin Med. Sch., Univ. Maryland Med. Sch., and Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Trypsin-trituration prepared primary cultures of brain, brainstem, and/or spinal cord were obtained from day 17 embryo, day 1 newborn, and day 10, 21, or 28 day post-natal outbred CD-1 albino or inbred Balb/C mice. Secondary cultures prepared at 1 or 5 weeks post plating contained 90-98% glial fibrillary antigen positive astrocytes and less than 0.5% galactocerebroside positive cells.

Infection of astrocytes with a cytolytic alphavirus--Sindbis virus--is much more efficient(10-100 x)with the neuroadapted strain than with the non-neuroadapted strain in embryonic and day 1 newborn astrocytes, but efficiency decreases significantly (100 x)with infection of day 10 or older post-natal astrocytes by the non-neuroadapted strain of Sindbis virus. Between 24-36 hours post infection the astrocyte cultures are completely destroyed. This age-related target cell resistance is probably located at the cell surface because the yield of infectious virus particles per infected cell is nearly identical for astrocytes from animals at all ages.

Infection of astrocytes with a non-cytolytic murine retrovirus --RNA Tumor virus--is much more efficient with the neurotropic strain(CasBr)than with the non-neurotropic strain(AKR). However, in astrocytes from day 10 post-natal mice there is an age-related restriction of productive virus replication(100 x)which is not present in fibroblasts from animals of the same age. This restriction is not at the cell surface because infectious center formation is identical in day 1 and day 10 astrocytes and the yield of infectious virus particles released from day 10 astrocytes is decreased. There is no cytopathic effect of neurotropic retrovirus infection on astrocytes but terminal cell density as well as population doublings decrease with subculture in infected astrocytes compared to uninfected astrocytes. Genetic restriction of neurotropic retrovirus replication in Balb/C astrocytes compared to CD-1 astrocytes is demonstrable in vitro.

Thus, age-related resistance to virus infection by neurotropic viruses can be recapitulated in astrocyte cultures in vitro. The target cell resistance is manifest in different ways with different viruses. Restriction of virus replication in astrocytes may take place at the initial interaction of the virus with the cell surface or be dependent on intracellular events not related to cell surface virus receptors.

62.15 EXPRESSION OF MYELIN PROTEOLIPID PROTEIN IN PRIMARY CULTURES OF FETAL RAT BRAIN W.B. Macklin* and S.E. Pfeiffer. Dept. Biochemistry, E.K. Shriver Center, Waltham, MA 02254 and Dept. Microbiology, U. Conn. Health Sciences Center, Farmington CT 06032

Several myelin markers, for example sulfatide synthesis, myelin basic protein (MBP) accumulation and 2',3' CNP and cholesterol ester hydrolase activities, have been studied in mixed primary cultures (MPC) of fetal rat brain. The quantitative expression of these markers approaches normal in vivo levels during differentiation of MPC. The present studies were undertaken to investigate the expression of proteolipid protein (PLP) in these cells. Quantitation of PLP has been complicated by its unusually hydrophobic nature and its tendency to aggregate and precipitate in aqueous solution. Nevertheless, the use of high titer PLP antiserum in the electroblot technique (Towbin et al. PNAS 76: 4350, 1979) permits immunologic identification of as little as 125 ng PLP in samples containing several hundred micrograms of protein. The present study indicates that, in contrast to the other myelin markers, the amount of PLP in these cells is greatly reduced. When single 60 mm culture dish samples containing 200-400 pmol MBP/plate were solubilized, electrophoresed and electroblotted with PLP antiserum, little or no PLP was detected. It was possible to detect PLP only after subcellular fractionation of a large number of cells. MPC were homogenized in 0.32 M sucrose and centrifuged at 12,000 x g for 20 min. The supernatant was then centrifuged at 100,000 x g for one hr while the pellet was applied to a discontinuous sucrose gradient containing 0.9 M and 1.2 M sucrose and centrifuged at 100,000 x g for one hr. Three fractions were isolated from the gradient: the 0.32/0.9 M sucrose interface, the 0.9/1.2 M sucrose interface and the pellet. These three fractions and the earlier 100,000 x g pellet were solubilized, electrophoresed and electroblotted. The only fraction which contained PLP was the 0.32/0.9 M sucrose interface. The PLP band in this fraction was compared by densitometry to an electroblot containing several known concentrations of PLP. By this analysis, this fraction, which contained 182 pmol MBP by radioimmunoassay, contained only 10 pmol PLP. Thus, PLP, rather than reaching a level greater than MBP as it does in vivo, is present in this fraction at approximately 5-6% the level of MBP. Since the concentration of PLP is extremely low in these cells and the cells make little or no detectable myelin, it is tempting to speculate that the signals regulating PLP expression are somewhat different from those regulating the other myelin markers and that they may be closely connected to the signals regulating actual myelin formation. Supported by grants NS10861, NS13649, NS16945 and F32NS06192 (WM) from NIH and RG1213 from Nat. M.S. Society.

62.16 IMMUNOCYTOCHEMICAL CHARACTERIZATION OF TWO CELL POPULATIONS GROWING FROM EXPLANTS OF MALIGNANT HUMAN GLIOMAS. B.M. Chronwall*, P.E.McKeever*, B.H. Smith and P.L.Kornblith, Surgical Neurology Branch, NINCDS, National Institutes of Health, Bethesda, MD 20205.

Fifteen 1mm^3 explants derived from 3 minced malignant human gliomas obtained at surgery have been characterized with respect to their cellular outgrowth pattern. After 1-3 weeks in culture, two different cell populations grew out. Their staining properties correlated with cell populations on frozen sections of intact glioma tissue which consistently showed fibronectin (FN) positive (+) GFAP negative (-) immunohistochemical staining of the vessel walls and GFAP+, FN- staining of the parenchyma. A mat of fascicular FN+, GFAP- cells with a polygonal, flat fibroblast like morphology covered the growth substrate (glass or plastic) in a swirling pattern for several mm around an FN+ explant. EM preparations showed dilated RER and extracellular filamentous strands which stained with HRP anti-FN. In contrast, around other explants, GFAP+, FN- cells resembling astrocytes grew in a net-like pattern with little cell to cell contact. Few of these cells were found more than one mm from an explant. EM preparations of GFAP+ cells contained abundant filaments in cell processes but lacked extracellular filaments and dilated RER. In mixed explants GFAP+ cells grew on top of FN+ cells. Individual cells did not stain for both GFAP and FN up to 3 weeks after explantation. Clones derived from the two populations are being utilized to further characterize and understand events during long-term culture.

62.17 HUMAN SCHWANN CELLS CULTURED FROM DISSOCIATED NERVE BIOPSIES. Jean R. Wrathall and Carl C. Kao*. Dept. of Anatomy, Georgetown Univ., and Veterans Admin. Med. Ctr., Washington, D.C. 20007.

We have previously investigated methods for the production of nonneuronal cell cultures from dissociated sensory ganglia (Brain Res. 229:163, 1981) and peripheral nerve segments (Brain Res. 224:118, 1981) of the adult cat. Methods for optimal tissue dissociation and for the production of cultures and cell lines highly enriched in Schwann-like cells were described. We have now investigated the application of these methods to the production of Schwann cell-enriched cultures from human peripheral nerve.

Biopsy specimens of the sural or greater occipital nerve were obtained from 5 patients who underwent neurectomy due to post-injury pain or other disorders. The epineurial and outer perineurial connective tissue was removed, the nerve fascicles minced, and the pieces used directly to establish explant cultures (Askanas et al., Arch. Neurol. 37:329, 1980) or subjected to enzymatic dissociation with solutions of trypsin, collagenase or a combination of these enzymes. Suspensions of dissociated nerve were cultured in collagen-coated flasks with modified Eagle's MEM and 10% fetal calf serum, as previously used for the feline cultures. The effect of differential attachment on the composition of the primary cultures was studied by sequential transfer of the suspensions to new culture flasks at 1, 4, 24, 48 and 72 hrs after dissociation.

The most vigorous primary cultures were obtained by incubation of the nerve pieces with 0.25% collagenase in HBSS at 36° for 2 hrs followed by mechanical agitation. The resulting suspension consisted of lengths of myelin sheath tubes as well as individual cells freed during dissociation. These individual cells (as well as some of the myelin segments) attached rapidly, within 1 hr after dissociation, and produced primary cultures that consisted mostly of very flat, multipolar cells. Most of the myelin segments attached more slowly, over the course of several days. Typical Schwann cells were observed to grow out from these segments of degenerating myelin sheath tube and formed chains of bipolar, spindle-shaped, phase-refractile cells. By 7-10 days after initiation, these cultures contained a network of bipolar Schwann cells and a small proportion of flat, multipolar, fibroblastic cells. Thus, differential attachment allowed the production of primary cultures enriched in Schwann cells. As with cells cultured from adult cat peripheral nervous tissue, these adult human Schwann cells continued to proliferate in the absence of neurons and could be subcultured while retaining the typical morphology of Schwann cells in culture. (Supported by the VA and Vinny's Spinal Cord Res. Found. of Rhode Island, Inc.)

62.18 AN IN VITRO MORPHOMETRIC STUDY OF GLIAL AND SCHWANN CELL MYELINATION--EXPRESSION OF AXONAL DIAMETER DEPENDS UPON MYELINATION. A.J. Windebank*, P. Wood*, R.P. Bunge, and P.J. Dyck, Mayo Clinic, Rochester, MN 55905, and Washington Univ. Sch. of Med., St. Louis, MO 63110

To evaluate morphometry in tissue culture, we have undertaken a comparative, quantitative, sequential study of myelination of dorsal root ganglion (DRG) neurons by central or peripheral supporting cells. This study also tests the hypothesis that differentiated features are expressed in culture. Dissociated DRG cultures from 15-day rat embryos, (E_{15}) free of Schwann cells and fibroblasts were prepared by previously described methods (Wood et al, 1980, Brain Res. 196:247). Supporting cells were added as fragments of E_{14} spinal cord or E_{16} DRG; myelination commenced after 2 weeks. Control cultures received no supporting cells. At 7, 14 and 24 days, a total of 22 cultures were processed for electron microscopy. Three fascicles from defined points were sampled from each culture. Qualitatitively, cytoarchitecture, ensheathment, extracellular collagen and basal lamina formation resembled the parent tissue. Myelin periodicity was 10.8±0.7nm for glia and 13.8±0.5nm for Schwann cells ($p<.001$); similar to values for CNS and PNS myelin in vivo. In cultures containing glial cells, smaller fibers ($p<.001$) were myelinated (mean of median diameter 1.13±0.13 μ) than in cultures containing Schwann cells (1.67±0.17μ) although there was no difference ($p>.1$) in the degree of myelination expressed as number of myelin lamellae/fiber. A new finding concerned the relationship of axonal diameter to the presence or absence of supporting cells. In control cultures without supporting cells or in areas where supporting cells were absent there was a unimodal distribution of neurite diameter. A mean of 795 fibers were counted from each of 15 fascicles from 13 different cultures. The range of neurite diameter (0.05-1.2μ) and the median diameter (mean of median 0.24±0.03μ) were similar at different times (7,14,24d), demonstrating a stable population of neurite diameters throughout the period. In 10 myelinated fascicles, a bimodal distribution of neurite diameters was present. The second peak represented myelinated neurites which had a greater median diameter (measured to inner border of myelin) and a different range of fiber diameters compared to bare neurites. For Schwann cells this range was 0.7-3.4 μ and the mean of median diameters was 1.67±0.17μ; for glial cells the range was 0.6-2.4 μ and the mean of median diameters 1.13±.13 μ. Differences between myelinated and bare fibers were all highly significant ($p<.001$). The absence of the larger diameter peak in fascicles containing only bare neurites suggests that myelination by Schwann cells or oligodendrocytes is necessary for the expression of axonal diameter greater than 1.2 μ in this tissue culture system.

62.19 UV LASER MULTISHOT TRANSECTION OF GLIAL AND NEURONAL CELL PROCESSES AT LOW ENERGY DENSITIES. J. H. Lucas*, H. Stewart*, L. Higgins* and G. W. Gross (SPON: J. F. Hines), Dept. of Biology, The Texas Woman's University, Denton, TX 76201.

Cell surgery utilizing a pulsed UV laser microbeam promises to be a useful tool for selective deletion of cells and processes from neuronal circuits in culture, for study of morphological alterations on single neuron function, for specific cell deletion in embryonic systems and for quantitative studies of surgical trauma and regenerative capacity. We have previously reported two single shot techniques that could be used effectively in culture a) direct cytoplasmic transection at 12 μj/μm^2, and b) indirect substrate shock-wave transection at lower energy densities. However, despite cell survival, concurrent TEM investigations have revealed cytoplasmic disruption and mitochondrial vacuolization beyond the laser impact area. As a possible means of minimizing damage during lasing, we have developed a high frequency multiple shot technique at energy densities below that required for single shot cytoplasmic vaporization. Surgery was performed on dissociated embryonic mouse spinal cord cells grown on glass cover slips to prevent substrate involvement during lasing. Transections of neuronal and glial processes were attempted at 337 nm, firing frequencies of 20 and 60 Hz and at an energy density of 5 μj/μm^2. Irradiation intervals ranged from 1 to 14 seconds. The relationship between the percentage of successful transections and the irradiation interval appears sigmoidal and requires 9 seconds at 60 Hz and 5 μj/μm^2 and 15 seconds at 20 Hz and 5 μj/μm^2 to achieve 100% transection. Preliminary EM data of ultrastructural changes at the target point and in the surrounding cytoplasm suggest greatly reduced damage compared to that found with single shot transection energy densities. Light microscopic observations reveal a slow cytoplasmic pinching followed by collection of material on either side of the target culminating in process transection within 30 seconds post lasing. The transection phenomenon is multifactorial and is influenced by process tension, adhesion, and diameter as well as by the presence or absence of a glial carpet. Preliminary observations reveal possible differences in the susceptibility to laser irradiation of neurons and glia and of cells from cultures at different stages of development. These factors affect the reproducibility of transection but also suggest that the pulsed UV laser may be used as a probe of basic cytoskeletal function, development and integrity. Supported by NIH Grant NS15167.

62.20 THE EFFECTS OF TEMPERATURE AND POTASSIUM ON THE MEMBRANE POTENTIAL OF RAT TAIL SMOOTH MUSCLE CELLS IN CELL CULTURE. H. J. Bryant, F. A. Kutyna, L. J. Lewis*, J. W. Patrickson*, M. B. Pamnani*, S. J. Huot*, and F. J. Haddy*. Dept. of Physiology, Uniformed Services University, Bethesda, MD 20814 and Cleveland Clinic, Cleveland, OH 44106.

Membrane properties of arterial smooth muscle cells in vivo and in vitro can be influenced by a variety of factors such as neural input, tension, ions and hormone concentrations. Because whole arteries contain several types of cells, it is difficult to insure that the measured membrane properties are exclusively those of smooth muscle. The use of a primary culture obviates many of these sources of error. The present study measured membrane potential as a function of temperature and external potassium concentration in cultured cells from Wistar rat tail arteries.

Standard culture procedures were used to produce monolayers of smooth muscle cells. Cells were grown in a modified GIBCO 199 medium for 7 to 10 days before recording. Intracellular membrane potentials were obtained by means of 40 - 100 megohm glass microelectrodes filled with 3M KCl. For the temperature study the preparation was constantly superfused with a Krebs-Henseleit (KH) solution containing 5.8 mM potassium. The potassium study used solutions containing from 0.1 to 100 mM potassium which were maintained at 37°C. All solutions were aerated with a 95%-5% oxygen carbon-dioxide mixture. Cultures were maintained on a temperature-controlled microscope stage and observed during the experiment using phase optics.

KH vs. 199

The membrane potential (E_m) of the cells in the standard KH solution was 61.8 ± 1.02 (X ± SEM, N=158). This value was hyperpolarized 20 to 30 mV compared to the E_m measured from cells in the tissue culture medium.

Temperature

As the temperature of the culture superfused with KH was decreased from 37°C to 15°C, the membrane depolarized to 33.9 ± 2.2 mV (N=37). The change in the membrane potential over the temperature range 17°C to 35°C was 1.4 mV/°C.

Potassium Concentration

Rhythmic action potentials were observed in some cultures superfused with normal KH and high potassium solutions.

Rat tail artery smooth muscle in primary cultures shows characteristics similar to those seen in vivo and in vitro preparations and may serve as a fundamental model for electrophysiological studies. (Supported by NIH Grant HL21525-05, USUHS CO7605 and CO7607).

62.21 ABSENCE OF MYELIN BASIC PROTEINS IN SHIVERER ORGANOTYPIC AND NORMAL-SHIVERER CONFRONTATION CULTURES OF MOUSE DORSAL ROOT GANGLIA.

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The Shiverer (shi) mouse has an autosomal recessive mutation which results in a gross deficiency in the amount and quality of myelin in the CNS but a normal amount in the PNS. The 14,000 to 19,000 dalton myelin basic proteins (MBP) are undetectable in either the PNS myelin or in the CNS of homozygotes (shi/shi). However, it is not established whether the Shiverer locus acts intrinsically in the nervous system or extrinsically, nor is it known what cell types are the primary cellular targets of the mutant gene. We have used tissue culture and immunocytochemical techniques to investigate these questions. In the first series of experiments organotypic cultures of dorsal root ganglia (DRG) from 17-day embryonic shi/shi or +/+ mice were grown on collagen in MEM supplemented with 15% human placental serum, 5% chick embryo extract, 600 mg/% glucose, and NGF. Within 5-6 weeks both cultures contained myelin in equivalent amounts. Presence of MBP in myelin was determined immunocytochemically. Whole cultures were fixed in $HgCl_2$-formalin (2.0 ml 30% formaldehyde and 7.6 ml saturated $HgCl_2$ in H_2O, 1 hr, 4°C), treated with 95% ethanol (20 min, 25°C), with 0.25% Triton X-100 (20 min, 25°C), with 3% normal rabbit serum (1 hr, 25°C), reacted with goat anti-rabbit MBP (provided by Dr. S. Cohen, 1/500 dilution in 0.5 M Tris-HCl, pH 7.6; 1% rabbit serum; 0.025% Triton X-100, 18 hr, 4°C) and then with rabbit anti-goat IgG labeled with horse-radish peroxidase (1/50 dilution in same diluent, 2 hr, 25°C). Whereas myelin in +/+ cultures stained positively for MBP, myelin in shi/shi cultures was negative. To narrow further the primary cellular target of the mutant gene a second series of experiments was carried out. Dissociated +/+ DRG were treated with 5-bromodeoxyuridine (10^{-5}M) for 3 days, then treated with Hoechst dye 33258 (5 µg/ml) for 3 hr, exposed to light for 1 hr (60W fluorescent, 12 cm above culture), and treated with fluorodeoxyuridine (10^{-5}M) for 3 days. This treatment removed >99% of non-neuronal cells and left apparently intact neurons with abundant neurites. To establish confrontation cultures of those neurons with mutant or control non-neuronal cells pieces of sciatic nerve from neonatal +/+ or shi/shi mice were introduced into the cultures. Three weeks later myelin had formed and immunocytochemical staining showed that cultures seeded with +/+ nerve produced MBP-positive myelin, while cultures seeded with shi/shi nerve were MBP-negative. These results indicate that lack of MBP in Shiverer PNS myelin does not result in gene action extrinsic to the PNS nor is it a primary affection of the sensory neurons: the Shiverer gene acts in Schwann cells and/or fibroblasts of the PNS. Additional experiments using isolated DRG neurons from +/+ mice co-cultured with enriched Schwann cells and/or fibroblasts from shi/shi mice are underway to localize further the primary cellular target of the Shiverer genetic locus.

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63.1 UPTAKE OF AMINES BY GLIA IN THE FROG FILUM TERMINALE: EFFECTS OF CORD TRANSECTION AND 5,6DHT. B. Haber, T. Ritchie and H. T. Hutchison, Marine Biomed. Institute, Depts. of Neurology, Pediatrics and Human Biological Chem. and Genetics, Univ. of Texas Med. Branch, Galveston, TX 77550.

The frog filum terminale (FT) is that portion of the frog spinal cord that is caudal to the last spinal root. The morphology of the FT and its utility as a normal preparation of glia has been described (Glusman et al, Brain Res., 1979). The uptake of γ-aminobutyric acid (GABA), serotonin (5HT), Norepinephrine (NE) and dopamine (DA) has been shown by radioautographic techniques to occur in the glia of the FT, via saturable high affinity transport processes. The pharmacology of these uptake processes is similar if not identical, to the transport processes for these neurotransmitter substances in brain slices and synaptosomes. The uptake of 5HT differs from that of GABA and NE and DA in that it is maximal in the conus medullaris (CM), an area immediately caudal to the last spinal root. The CM is rich in fibers that are 5HT immunoreactive, thus suggesting that some of the 5HT uptake occurs in 5HT fibers and terminals in the neuropile of the CM. The FT contains only a narrow band of 5HT immunoreactive fibers, thus making it unlikely that the uptake of 5HT, NE and DA in the FT occurs into sites other that glia. Surgical transection of the spinal cord below L-10 and allowing ten days for degeneration to occur, results in a small reduction of GABA uptake in the FT, and a larger reduction in the CM. Intraventricular injections of the Neurotoxin 5,6 dihydroxytryptamine abolishes all 5HT immunoreactivity in all portions of the spinal cord, but has no effect on the uptake of GABA in either the CM of FT. Transection has little effect on 5HT uptake in the FT, but reduces it markedly in the CM. Use of the neurotoxin however, does reduce the uptake of 5HT in both FT and CM. Norepinephrine uptake is dramatically reduced in both FT and CM by transection, but not be 5,6DHT. We, therefore, conclude that 5HT uptake in the CM occurs into both glia and 5HT fibers and terminals, but occurs exclusively into glia in the FT. NE and DA uptake probably occurs largely into glia and less so into 5HT fibers and terminals. Taken together, these data indicate that the glial uptake of GABA, 5HT and the catecholamines is affected by changes in the cellular millieu that may result from the surgical or pharmacological lesioning of the descending inputs to the CM and FT.

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63.2 ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION OF GLIA IN THE FILUM TERMINALE OF THE FROG. M. Chesler and C. Nicholson. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, N.Y. 10016

The filum terminale of the frog constitutes the caudal spinal cord and contains mostly astroglial cells (Gonzalez-Robles, A. and Glusman, S., Cell Tiss. Res., 199: 519, 1979). This preparation, therefore, provides an unusual opportunity to study glia without neuronal influences. We have further characterized these cells using intracellular recording and staining techniques. The electrophysiological data are consistent with a glial categorization. HRP staining revealed cells lining the central canal and more peripheral elements of diverse morphology.

The filum from R. pipiens and R. catesbeiana was dissected and studied in-vitro as a whole or hemisected cord. Preparations perfused with oxygenated Ringer were viable up to 18 hrs. Stable penetrations were made throughout the filum and frequently maintained for 30 min. or more. Resting potentials ranged from 55-87 mV. negative (mean=70mV, n=105). Input resistance was 5-25 MΩ (mean=12 MΩ , n=14). The electrotonic response to a current step had a time constant of under 500μ sec. Such cells never gave injury discharges upon impalement (however, excitable elements, presumably axons, were occasionally impaled). The relationship between membrane potential and $[K+]_o$ was non-Nernstein probably because of the shunting effect of an incomplete electrode seal. No electrophysiological parameters were correlated with electrode location - cells could only be distinguished histologically. Cells were injected with HRP and the DAB-processed tissue was viewed in 250-400 μm transverse sections. Cells varied in size and shape, displaying unipolar, bipolar and multi-polar somata (7-15 μm diameter). Some cell bodies showed spinose excrescenses while others were smooth. Radially oriented processes were commonly seen running a tortuous path to form sub-pial end-feet. Canal cells were elongated, tapering towards the central canal with radially oriented processes similar to peripheral cells. A similar distribution of astrocytes has been described in the bullfrog (Sasaki, H. and Mannen, H., J. Comp. Neurol., 198: 13, 1981) and toad spinal cord (Stensaas, L.J. and Stensaas, S.S., Z. Zellfor., 84:473, 1968) using the Golgi method. Our HRP staining apparently revealed a greater variation in cell form than the Golgi studies. This may be due to a different sampling of cells or a more complete staining of glial processes using HRP. Supported by USPHS Grant #NS13742.

63.3 ELECTROPHYSIOLOGICAL STUDIES ON EPENDYMAL CELLS OF THE TURTLE CORTEX. B.W. Connors and B.R. Ransom, Dept. of Neurology, Stanford University School of Medicine, Stanford, CA 94305.

Ependymal cells are specialized neuroglia which line the ventricles of the brain. Very little is known about their function or electrophysiological properties. In the visual cortex of the turtle Pseudemys scripta the ependymal cells extend radial processes from their cell bodies lying at the ventricular wall to the outer surface of the brain, where they form a limiting glial layer under the pia. At the ultrastructural level, some ependymal cells in this preparation appear to receive axo-glial synaptic contacts (Ebner & Colonnier, J. Comp. Neurol. 160:51, 1975). In our studies the visual area of cortex was dissected free, placed with the ventricular surface upward in a recording chamber and bathed in an oxygenated turtle saline with $[K^+]_o$ of 2.6mM. Intracellular recordings obtained from ependymal cells revealed high resting membrane potentials (-90 ± 4.5mV; mean ± S.D.), electrical inexcitability and no obvious spontaneous or evoked synaptic potentials. To assess the relationship between $[K^+]_o$ and membrane potential, the bath $[K^+]_o$ was increased while simultaneously recording from an impaled cell. Changes in $[K^+]_o$ were continuously monitored in the adjacent extracellular space via K^+-specific microelectrodes. Cells studied in this manner had a slope of 53mV for a 10-fold change in $[K^+]_o$, suggesting that K^+ was the predominant ion responsible for the resting potential. Ependymal cells responded with slow depolarizing potentials up to 48mV in amplitude during repetitive local cortical stimulation. By monitoring changes in $[K^+]_o$ it was possible to infer that these slow depolarizing potentials were mediated entirely by increases in $[K^+]_o$. Injection of Lucifer yellow CH into single ependymal cells resulted in the staining of a two dimensional array of adjacent ependymal cells. The injected cell was brightly stained, and its radial process was often identifiable. The intensity of staining in successive rings of dye-coupled cells fell off steeply. By simultaneously recording from two closely spaced cells it was possible to demonstrate the passage of current from one cell directly into the other, thereby confirming the presence of electrical coupling suggested by the widespread dye-coupling. When the pH of the bathing solution was decreased (from 7.4 to approx. 6.9) by increasing the concentration of ambient CO_2, dye-coupling was abolished. These observations indicate that ependymal cells in turtle cortex behave very much like glial cells studied elsewhere, but leave unanswered the functional significance of the observed synaptic contacts and the radial processes.

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63.4 CORRELATION BETWEEN FLUXES OF K^+ AND OF Cl^- IN ASTROCYTES. W. Walz and L. Hertz*. Dept. of Pharmacology, University of Saskatchewan, Saskatoon, S7N OWO, Canada.

Transport systems operating in astrocytes were characterized by measuring unidirectional fluxes of ^{42}K and ^{36}Cl. The astrocytic preparations used were 28-40 day-old pure primary cultures prepared from newborn Swiss mice, grown in a modified MEM, and from the age of 2 weeks in the additional presence of 0.25 mM dBcAMP. All transport measurements were made with cultures in the steady-state with respect to ion composition. The total K^+ uptake rate (measured during a 10 sec period) was 2025 nmol mg^{-1} protein min^{-1}. This rate was not influenced by furosemide (2 mM) or acetazolamide (0.1 mM); ouabain (1 mM) inhibited the uptake rate by 26.7% or 541 nmol mg^{-1} min^{-1}. The equilibrated K^+ content was determined to be 777 nmoles mg^{-1}. The efflux rate of K^+ was determined using a washing efflux method (Boonstra et al., Biochim. Biophys. Acta 643: 89, 1981). The rate constant for efflux was 2.65 min^{-1}. This equals a halftime of 16 sec for exchange of the initial K^+ pool. The rate constant was not significantly altered by furosemide (2.66 min^{-1}, halftime 16 sec) or by ouabain (3.5 min^{-1}, halftime 12 sec).

The equilibrated Cl^- content was found to be 167 nmol mg^{-1} The total Cl^- uptake was 35 nmole mg^{-1} (measured during a 2 min period). 68% was inhibited by furosemide, 40% by SITS (1 mM); both together inhibited 79% of the uptake, leaving 7 nmol min^{-1} mg^{-1} as non-inhibitable.

The ratio between the non-inhibitable K^+ and Cl^- uptake rates (which are likely to be non-carrier mediated and therefore diffusional) is 212:1. It can therefore be concluded that the cell membranes of the cultured astrocytes are highly and selectively permeable for K^+, making these cells suitable for function as spatial buffers. These qualitative and quantitative results are consistent with the findings on mammalian astrocytes in situ (Trachtenberg and Pollen, Science 167: 1248, 1970; Lothman and Somjen, J. Physiol. 252: 115, 1975). In addition, there is a considerable ouabain-sensitive part of the total K^+ uptake, which does not represent a K^+-K^+ self-exchange (since the efflux is not affected by ouabain). This ouabain-senstitive uptake rate is enhanced threefold when the external K^+ concentration is increased to 12 mM, as we have shown for K^+-depleted, non-steady-state astrocytes (Walz and Hertz, J. Neurochem., in press). Our experiments point out 1) that the astrocytes in primary cultures have similar K^+ and Cl^- transport properties as mammalian astrocytes in situ and 2) that two uptake systems (spatial buffer mechanism and active K^+ uptake by a Na^+-K^+ ATPase) combined could well account for the observed clearance rates of the extracellular K^+ in the mammalian brain. (Supported by the MRC of Canada and Deutsche Forschunggemeinschaft).

63.5 SULFATE TRANSPORT AS A MODEL FOR CHLORIDE TRANSPORT IN GLIA CELLS
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Astrocytic membrane transport systems play a significant role in regulating the composition of extracellular fluid in brain. Our recent work [Trans.Am.Soc.Neurochem. 13:134(1982)] indicates the existence of at least two mechanisms for Cl^- transport in the rat glioma cell line, LRM55. One mechanism, similar to the Band III protein in erythrocyte membranes, appears to exchange intra- and extracellular Cl^-. The other mechanism, which can be stimulated by elevated $[K^+]_o$, may involve cotransport of K^+ and Cl^-. These findings are consistent with the characteristics of Cl^- transport in rat primary astroglia [H.K. Kimelberg, Biochim.Biophys.Acta. 646:179-184(1981)]. In the work presented here, we have studied $SO_4^=$ as a transport analogue for Cl^- in cultures of LRM55 cells. We sought to determine whether $SO_4^=$, which is a good substrate for the erythrocyte anion exchanger, is selectively transported by the glial anion exchanger and not by other anion porters available to Cl^- in glia.

In HEPES-buffered isotonic salt solution containing 7 mM Cl^-, the time course of $^{35}SO_4^=$ uptake was biphasic; there was a rapid initial influx of $SO_4^=$ followed by efflux to reach the steady state concentration. In the presence of higher concentrations of Cl^- in the incubation medium, the initial rate of $SO_4^=$ uptake decreased. When intracellular $[Cl^-]$ was first reduced by preincubation in 7 mM Cl^-, the initial rate of $SO_4^=$ uptake decreased to 41% of control. We tested the effects of furosemide and SITS (4-acetamido-4'-isothiocyano-2,2' stilbene disulfonate) on $SO_4^=$ uptake, since these drugs caused partial inhibition of Cl^- influx in LRM55 cells. At 1 mM SITS or 5 mM furosemide, uptake of $SO_4^=$ was inhibited by more than 95%, which contrasted sharply with the lower efficacy of these drugs on Cl^- uptake (41-65% and 73-82% inhibition, respectively).

The present results suggest a relationship between $SO_4^=$ transport and Cl^- transport in LRM55 cells. Since uptake of $SO_4^=$ was inhibited by external Cl^-, these two anions may be competing for a common transport site. Reduction of $[Cl^-]_i$ caused a decrease in uptake of $SO_4^=$, which suggests that $SO_4^=$ may be taken up via an anion exchanger that accepts either anion. Since $SO_4^=$ uptake was completely inhibited but Cl^- uptake only partially inhibited by SITS or furosemide, Cl^- may have one or more routes across the membrane which are not available to $SO_4^=$. Thus, in these glioma cells, $SO_4^=$ may provide a tool for selectively studying one Cl^- transport system, to the exclusion of others.
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63.6 EFFECTS OF K^+, Na^+ AND Cl^- ON MEMBRANE POTENTIALS AND I-V CURVES OF PRIMARY ASTROCYTE CULTURES.
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We have reported (Kimelberg et al. Brain Res. 177:533-550, 1979) that astrocytes in primary cultures started from neonatal rat brain have membrane potentials of -65 to -75mV. They show a close to Nernstian response to external K^+ ($[K^+]_o$) down to values for $[K^+]_o$ of 4mM and have an average intracellular $[K^+]_i$ of 130mM. In the present study the perfusion of such cells with K^+-free medium resulted in an initial hyperpolarization of around 30mV followed by a progressive depolarization, presumably due to loss of K^+ and gain of Na^+. Such cells were impaled with 2 electrodes and current-voltage (I-V) curves were obtained after increasing times in K^+-free medium. There was a progressive decrease in conductance from 500 to 32nS at 23 mins after addition of K^+-free medium. We fitted these results to a computer program (C. Bowman and A. Baglioni, JCB 91:259a, 1981) applying the Goldman current equation to values for I and V. We found that we could obtain a constant value for the K^+ permeability coefficient (P_K) assuming that $[K^+]_i$ fell to 10mM after 23 min. The I-V curves could not be fitted assuming a permeability to K^+ alone but were fitted by assuming some additional permeability to either Cl^- or Na^+ using intracellular values of 35 and 20mM respectively. Values obtained were $P_K = 1.4 \times 10^{-4}$, $P_{Cl} = 9.6 \times 10^{-6}$ and $P_{Na} = 1.9 \times 10^{-6}$ cm sec^{-1} based on a value for the area of a spherical 40μm diameter cell of 5×10^{-5} cm^2. We found gap junctions in our cells by scanning electron microscopy, and also observed intercellular transfer of lucifer yellow. Thus, the effective area of the glial syncytium could be larger than a single cell. Based on the single cell area an input resistance of 2MΩ would give an area-specific resistance of 100Ω.cm^2. However, from oscilloscope recordings of the time courses of the induced voltage change during the I-V experiments we obtained time constants of 2 to 3msec, giving values of 2000 to 3000Ω.cm^2 based on a value of 1μF.cm^{-2} for membrane capacitance. This implies a surface area of the glial syncytium of 1×10^{-3} to $2.5 \times 10^{-3} cm^2$, requiring a coupling of about 20-30 astrocytes (ignoring the contribution of cell processes) and reducing P_K to a value in the range of 2.8 to 7 x 10^{-6}cm sec^{-1}. The effects of omission of Na^+ or Cl^- on the membrane potentials of these cells supported the concept that the secondary ionic conductance is to Cl^-. Omission of Na_o^+ did not hyperpolarize the cells, but did lead to a delayed depolarization. Omission of Cl_o^- usually resulted in a small, immediate depolarization of 2-3mV, but occasionally depolarizations of up to 20mV were seen. (Supported by NINCDS grant NS 13042).

63.7 ACTIVATION OF [^{3}H]2-DEOXYGLUCOSE UPTAKE IN GLIAL CELL CULTURES.
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The contribution of glial cells to the rate of energy metabolism in the CNS during various types of activity as measured by the deoxyglucose method (Sokoloff et al., J. Neurochem. 28:897, 1977) has not been determined. We have used cell culture to study cues which activate glucose utilization by glia and to examine the role of (Na,K)-ATPase in mediating such activation.

Mixed cell cultures of astrocytes and oligodendrocytes were prepared from the cerebral hemispheres of newborn mice by methods similar to those of McCarthy and deVellis (J. Cell Biol. 85:890, 1980). Uptake of [^{3}H]2-DG tracer was measured during 5-20 min incubation periods (34°C) in modified Hanks' balanced salt solutions containing glucose 5 mM. When 2-DG uptake was compared in replicate cultures bathed in solutions containing different K^+ concentrations, uptake was 2.4 - fold (± 0.2 S.E., N = 5) greater at 2 mM K^+ than at 0.4 mM K^+. Raising $[K^+]$ in the physiological range above 2 mM did not always elicit a significant further increase in 2-DG uptake (compare Cummins et al., Brain Res. 170:190, 1979). There was evidence that factors such as sodium-loading and bicarbonate concentration may modulate the potassium dependency of 2-DG uptake. After sodium-loading by pre-incubation of the cultures for 3 hr in a low $[K^+]$ solution (0.4 mM) 2-DG uptake was 4-fold greater at 20 mM K^+ than at 2 mM K^+. Also, 2-DG uptake doubled when bicarbonate concentration was raised from 4.3 mM to 50 mM in HEPES-buffered solutions (pH 7.2).

The ionophore monensin was used to catalyze sodium accumulation (Lichtshtein et al., PNAS 76:2580, 1979). In the presence of monensin 20 μM ($[K^+]$ = 5.8 mM) glial 2-DG uptake was increased by a mean factor of 3.1 ± 0.1 (S.E., N = 7). This effect of monensin was blocked by ouabain 0.4 mM. The effect of increased $[K^+]$ up to 20 mM responded inconsistently to ouabain 1 mM.

Thus, it appears that sodium-loading of glia by monensin causes a marked increase in glucose utilization that is mediated by activation of (Na,K)-ATPase. The set-point for the effect of $[K^+]$ on 2-DG uptake may be dependent on intracellular $[Na^+]$ and other as yet undetermined constraints. The extent of involvement of (Na,K)-ATPase in the $[K^+]$ effect remains to be established. (Supported by USPHS grant MH29011, U.S. Army contract DAMD 17-81-C-1279 and NSF grant BNS 19481.)

63.8 EFFECTS OF TRIETHYLTIN ON MEMBRANE TRANSPORT BY GLIOMA CELLS.
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Glial swelling or intramyelinic edema, a result of triethyltin (TET) intoxication, may result from an impairment of membrane transport processes in glia and an accompanying osmotic imbalance across the glial membrane. We examined the effect of TET on the transport of [^{3}H]taurine, [^{3}H]glutamate, $^{22}Na^+$, $K^+(^{86}Rb^+)$, and $^{36}Cl^-$ by LRM55 glioma cells in culture. TET was a potent inhibitor of taurine transport (IC_{50} = 3.0 μM) but a much less effective inhibitor of glutamate transport (IC_{50} = 132 μM). Low concentrations of TET (<10 μM) had only slight effects on $^{22}Na^+$, $^{86}Rb^+$, or $^{36}Cl^-$ transport. Much higher concentrations (1 mM) of TET appeared to cause a large, non-specific increase in membrane permeability and a corresponding increase in the transmembrane movement of $^{22}Na^+$, $K^+(^{86}Rb^+)$, $^{36}Cl^-$ and taurine.

The effect of TET on taurine transport was studied in more detail. Preincubation of cells with TET before addition of [^{3}H]-taurine showed that TET acted rapidly and that its effects could be reversed by incubation in TET-free medium for 10 min. Kinetically, inhibition by TET was due to its effect on the V_{max} for taurine transport; TET (5 μM) reduced the V_{max} from 2.09 nmol taurine/min/mg prot in the control to 0.55 and reduced K_m for taurine (an activating effect) from 37 to 18 μM. Comparison of the effects of TET and ouabain (which increased internal $^{22}Na^+$ about 4-fold but only slightly (<20%) inhibited taurine transport) indicated that TET did not inhibit taurine transport through any possible effects on the Na^+/K^+-ATPase. Tributyl- and tripropyltin (IC_{50} = 4.5 and 1.3 μM respectively) were about as effective inhibitors of taurine transport as TET but trimethyltin (IC_{50} = 145 μM) was much less effective.

These findings demonstrate that triethyltin may selectively inhibit taurine uptake by glial cells. This effect is not produced by the non-specific disruption of membrane permeability observed with triethyltin at higher concentrations.
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63.9 CHANGES IN ASTROCYTIC PLASMA MEMBRANES AFTER AXOTOMY. J.R. Wujek and P.J. Reier. Dept. of Anatomy, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Rectilinear aggregates of intramembranous particles (i.e., orthogonal arrays) are a characteristic feature of mammalian astrocytes as visualized with the freeze-fracture method. These arrays are typically concentrated at the glia limitans, whereas they are much less prevalent in astrocytic membranes within the parenchyma of the CNS. Recent studies (Anders and Brightman, J. Neurocytol. 8: 777, 1979) have shown, however, that in a moderate gliosis, induced by non-traumatic neural tissue transplantation procedures, the density of orthogonal arrays increased on astrocytic membranes subjacent to the glia limitans. In the present study, we have used the freeze-fracture method to determine: (1) if comparable membrane alterations occur in glial scars formed after axotomy and (2) how rapidly these changes evolve during the course of Wallerian degeneration. For these studies, glial scars were formed in the optic nerves of neonatal rats (5-8 days old) by enucleation; these animals were allowed to survive for 3, 12, and 24 hrs and 2-4 months. Neonatal animals were used since axotomy at this postnatal age results in the formation of a dense scar which consists predominantly of astrocytes. In non-enucleated neonatal and adult littermates, the astrocytes within the parenchyma exhibited relatively few orthogonal arrays; the highest densities were localized primarily in the membranes of astrocytic endfeet of the glia limitans. In either region, the astrocytes of neonates possessed fewer arrays than those of adults. At 2-4 months post-axotomy, astrocytic membranes located within the parenchyma of the nerve exhibited a three-fold increase in array density after axotomy ($184\pm49/\mu m^2$) compared to adult controls ($53\pm23/\mu m^2$). In contrast, the density of orthogonal arrays in the astrocytic endfeet of the glia limitans remained virtually unchanged ($508\pm110/\mu m^2$). The onset of these membranous changes were investigated in the short-term animals. Five-fold increases in the density of orthogonal arrays in astrocytes within the parenchyma appeared as early as 3 hrs after axotomy ($87\pm45/\mu m^2$) compared to neonatal controls ($15\pm6/\mu m^2$). These results demonstrate that pronounced changes occur in astrocytic membrane structure during gliosis, as exemplified by the increase in orthogonal array density. In addition, these membrane alterations occur very rapidly in response to axotomy in immature animals, such that the densities of arrays at 3 hrs post-axotomy even exceeded the values of adult controls. We are currently investigating whether a comparable rapid response to axotomy occurs in mature animals. (Supported by NIH Grant NS 13836).

63.10 ASTROCYTIC RESPONSE TO CNS INJURY: AN IMMUNOHISTOCHEMICAL STUDY James R. Connor and Alan Peters, Dept. of Anatomy, Boston University Medical School, Boston, MA. 02118

An antibody to glial filaments was used to determine the response of astrocytes to a knife-cut in the cerebral hemisphere of three month old rats. A parasagittal knife-cut was made through the cerebral cortex along the entire extent of the corpus callosum and into the underlying hippocampus. Animals were allowed to survive for 2 or 8 days after the lesion. The rats were perfused through the aorta with a solution of 4% paraformaldehyde and 0.1% glutaraldehyde. Coronal sections at the level of the visual cortex were cut on a Vibratome at a thickness of 50 µm. The sections were reacted with GFA antiserum (1:1000) for 48 hours. The GFA anti-serum was generously provided by Dr. L.F. Eng. The PAP method of Strenberger was used for visualization of the antibody. This method reveals a distinct time-dependent astrocytic response to the injury. By two days post-lesion, the density of the antibody reaction in astrocytes proximal to the blade path was greatly increased compared to that in the contralateral hemisphere. The increased reaction has been previously termed fibrillogenesis. The fibrillogenesis observed in this study extended 400 µm from the blade path in the superior half of the cortex and 500 µm in the deeper half. In the white matter and hippocampus, fibrillogenesis was observed 750 µm away from the blade path. In addition to fibrillogenesis, there was an increase in the number of astrocytic somata and filament containing processes in the vicinity of the lesion. There were also indications of astrocytes undergoing cell division locally. Astrocytes containing debris were observed both near the lesion and surrounding nearby blood vessels. By eight days post lesion, the fibrillogenesis extended farther from the blade path and when compared with the two day survival group the astrocytic somata were larger and many had multiple large vacuoles in their cytoplasm. Astrocytes with many small vacuoles could also be observed. Indications of locally dividing astrocytes were also found in the eight day survival group. It is clearly possible to study the astrocytic response to CNS insult with immunohistochemistry. This investigation will be expanded to include: (i) longer post-lesion survival times, (ii) an electron microscopic evaluation, and (iii) different age groups to determine if the time dependent response to the lesion is also age dependent.

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63.11 SOMATOSTATIN POTENTIATES β-ADRENERGIC RESPONSE OF ASTROCYTES IN VITRO. G. Rougon*, M. D. Noble* and A. W. Mudge* (SPON: P. H. Patterson), MRC Neuroimmunology Project, Department of Zoology, University College London, Gower Street, London WC1E 6BT

With the view that neuropeptides may have a role in the functioning of the nervous system other than that of altering neuronal excitability, we have been studying the effect of some peptides on the physiology of astrocytes in vitro. Cultures of astrocytes derived from the cerebral cortex of newborn rats used in this study were 95% pure by the criterion of immunofluorescence-labelling of the glial specific intermediate filament protein-glial fibrillary acidic protein (GFAP).

When added to cultures of astrocytes norepinephrine (10^{-7}M) increased intracellular cyclic adenosine monophosphate (c-AMP) approximately 20-fold over basal levels in five minutes; this increase was inhibited by the β-antagonist propranolol. When the peptide somatostatin (at 10^{-7}M) was added together with the norepinephrine, c-AMP was increased approximately 50-fold over basal levels in five minutes. However, the addition of somatostatin (10^{-7}M) alone in the absence of norepinephrine did not alter basal c-AMP.

This result adds to the suggestion that neuropeptides may influence glial cells as well as neurons and provides another example of a neuropeptide influencing the response induced by another chemical signal without a detectable action of its own.

63.12 NEURONS MAINTAINED IN CULTURE CAN BE POSITIVELY IDENTIFIED WITH MONOCLONAL ANTIBODY A2B5. A.K. Hodson* and R.Y. Curbeam*. (SPON: D.B. Sanders) Dept. of Pediatrics, Duke Univ. Med. Ctr., Durham, N.C. 27710

Positive identification of neurons and glia derived from the developing brain is of utmost importance in the investigation of in vitro neuron-glial interactions. Morphological and biochemical parameters of bulk isolated enriched cell fractions are not sufficiently specific to positively identify individual cell types in vitro. Because tetanus toxin, which binds to gangliosides in neuronal membranes has been used as a neuron marker, we studied the binding of monoclonal antibody A2B5 which binds to GQ ganglioside (Eisenbarth, et al., Proc. Natl. Acad. Sci. U.S.A. 76:4913-4917, 1979) to neurons from neonatal rat brain maintained in vitro.

Primary cultures of whole rat forebrain were established by decapitating newborn pups and dissecting the forebrain free of meninges. The tissue was finely minced in phosphate buffered saline, trypsinised (0.25%) for 15 min, triturated and filtered through nylon screens. After 10 days in culture the primary mixed cultures were vertically vibrated and then exposed to rotary shaking. The cells in the supernatant were placed on polylysine coated coverslips and maintained in culture with Dulbecco's MEM with added insulin, 20% fetal calf serum, penicillin and streptomycin. For surface labelling the live cells on coverslips were exposed to one or both of the following antisera: galactocerebroside (Gal-C), oligodendrocyte antigens O1 and O4, tetanus toxin (TT), A2B5 and fibronectin (FN) followed by goat anti-mouse or goat anti-rabbit fluorochrome labelled IgG and fixed in acid ethanol. In double-labelling experiments with GFAP the cells after surface labelling were pre-fixed in acid ethanol and then exposed to fluorochrome labelled goat anti-rabbit IgG. Cells staining positively with A2B5 and TT had the characteristic cytological features of neurons. The small round cell somata had large vesicular nuclei and distinct nucleoli. The extensive arborisations of the dendrite-like processes showed a wide variety of patterns 150-250 microns with terminal expansions, dendritic spines, and varicosities. In this system with double-labelling technique tetanus toxin positive cells were positive with A2B5. No cells which stained positively for A2B5 reacted positively to GFAP, Gal-C, O1, O4 or FN. Monoclonal antibody A2B5 shares the surface marker properties of tetanus toxin for neurons maintained with mixed glial cultures. A2B5 is readily available in large quantities for the identification, isolation, and in the presence of complement, the elimination of neurons from mixed populations of bulk isolated cells.

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63.13 IMMUNOHISTOCHEMICAL VISUALIZATION OF FIBRONECTIN AND LAMININ IN ADULT RAT PERIPHERAL NERVE AND PERIPHERAL NERVE CELLS IN CULTURE. C. Cornbrooks, D. Carey*, R. Timpl*°, J. McDonald*+ and R. Bunge. Washington Univ. Sch. Med., Depts. Anat. & Neurobiol. and Internal Med.+ St. Louis, MO and Max Planck Inst. Biochim.°, Munich, W. Germany

The development of peripheral nerves requires that three major cell types [neurons (N), fibroblasts (Fb) and Schwann cells (S)] cooperatively relate to one another and an extracellular matrix (ECM). In order to understand this functional interdependence, it is important to identify the cell types and their biochemical components. We have examined the disposition in peripheral nerve of fibronectin (FN) and laminin (LAM), two components known to mediate cell-ECM interactions in numerous non-neural systems. Indirect immunohistochemical staining with affinity purified antibodies against FN and LAM at the light microscopic level on 10μ, frozen, unfixed cross sections of adult rat sciatic nerve revealed that FN was localized in a diffuse pattern throughout the endoneurium, perineurium and epineurium. LAM was localized in a discrete ring around each S-N unit and in the perineurium of the nerve. Neither antibody recognized pure populations of dorsal root ganglion N in culture. FN, recognized as a Fb synthesized macromolecule was characteristically present in live cultures associated with Fb but did not recognize live S in culture, regardless of their stage of differentiation (including myelination). In contrast, a pure (live) population of S was stained in a patchy pattern by the anti-LAM antibody. Staining of a pure population of peripheral nerve Fb for LAM was barely visible above background. S in contact with N in defined media (which proliferate but do not differentiate to ensheathe axons) maintained a spotty pattern of LAM distribution. On the other hand, S-N preparations in serum-embryo extract supplemented medium (which fully differentiate) were intensely stained in a linear pattern identical to the distribution of basal lamina surrounding ensheathed and myelinated axons. Immunoprecipitations with the FN and LAM antibodies against the radiolabeled peptides present in the culture medium from fully differentiated S-N cultures revealed bands on SDS-PAGE in the appropriate molecular weight regions of 220K (FN) and 250K/440K (LAM). From these data we conclude that both FN and LAM are components of the mature PNS. Antibodies to FN clearly delineate Fb populations but do not qualitatively stain S at the light microscopic level in any stage of differentiation, although S apparently produce small amounts of FN. In contrast, LAM is clearly synthesized by S in all stages of differentiation, including the stages prior to the morphological appearance of the S produced basal lamina, and thus may serve to delineate S from other PNS cells. Supported by NIH Grants NS09923, GM28002 and HL26009.

63.14 GFAP IN RAT LIVER IN KUPFFER CELLS? A.L. Gard, F.P. White and G.R. Dutton, Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, IA 52242.

The expression of glial fibrillary acid protein, a 49-51kd intermediate filament subunit protein, has been reported to be restricted to the central nervous system, specifically to the cytoskeletons of astrocytes, radial glia, ependymal cells, Müller cells and tanycytes.

Here we report on the apparent labelling of the stellate-shaped Kupffer cells in rat liver using various rabbit antisera raised against bovine and human GFAP, kindly supplied by other investigators. Specific labelling of the radiating cytoplasmic processes and perinuclear region was observed by indirect immunofluorescence and immunoperoxidase labelling of 8 μM cryostat sections of liver from neonatal and adult Sprague-Dawley rats. Specific labelling in liver and cerebellar thin sections was abolished by either omitting the primary antibody or absorbing the antisera with human brain GFAP (gift of L.W. Lapham). Immunoblotting studies were carried out on proteins from liver and brain homogenates transferred to nitrocellulose paper following separation by SDS-polyacraylamide gel electrophoresis. In brain, anti-GFAP sera recognized a prominent band at 49-50kd and a minor band, reported to be a GFAP degradation product, at 39-40kd. In liver, in addition to a faint band at 50kd, another band was labelled at 59-60kd.

We have yet to determine whether the specific binding in the liver is to authentic GFAP, similar molecular weight proteins with shared antigenic determinants, or an impurity common to the antisera that we have used.

The regulation of cell shape has been proposed as a function for intermediate cytoskeletal filaments. Therefore, the coincident morphological similarities of astrocytes and Kupffer cells may reflect the conservation of a GFAP-like protein in these cell types.

This work was supported by NIH training grant GM 07069, USPHS grant NS 16518 (GRD) and MRC Canada grant MT-5405 (FPW).

63.15 A MONOCLONAL ANTIBODY AGAINST AN INTERMEDIATE FILAMENT ANTIGEN LOCALIZED IN A SUBSET OF RAT ASTROCYTES, IN MENINGES, AND IN MUELLER CELLS OF THE RETINA. S. K. R. Pixley* and J. de Vellis (SPON: L. Kruger). UCLA School of Medicine, Los Angeles, CA 90024.

A monoclonal antibody was developed using a purified in vitro population of rat cerebral astrocytes as the antigen source. The indirect immunofluorescence technique reveals that the antibody (an IgMk subclass) binds to cultured cells which double label with anti glial fibrillary acidic (GFA) protein serum. GFA-negative cells in the culture also stain. A filamentous pattern of binding is seen in the cells that is almost identical to that seen with anti-GFA antiserum. The filaments bound by both antibodies coalesce into perinuclear bundles after colchicine treatment of the cells, indicating that these filaments are of the intermediate size class.

Fresh frozen cryostat sections of adult rat and mouse cerebellum show binding of the monoclonal antibody to fibers radially crossing the molecular layer, to meningeal cells, and to some large blood vessels in other layers. The molecular layer fibers co-label with anti-GFA antiserum, showing an astrocytic nature, probably Bergmann Glial Fibers. Staining in the rat retina is confined to cells having the characteristics of Mueller cells. Very little staining is seen in the adult rat neocortex other than a subependymal plexus and the meninges.

In the developing rat, at postnatal days 5 through 21, astrocyte-like cells in all layers of the cerebellum are stained, thus indicating that the antigen becomes restricted in distribution during development. Developmental regulation of the antigen is also observed in the telencephalon. In the cortex and hypothalamic area, fibers which radiate outward from the ventricles to the pia are stained at postnatal day 5, but disappear by day 15. Ependymal cells stain at day 15 and 21 but they do not stain at day 5 or in the adult. The meninges stain throughout development. Despite meningeal binding this antibody should prove useful for the study of subpopulations of glia and their differentiation.

The antigen(s) is currently being characterized by immunoblotting techniques and its subcellular distribution determined by immunoperoxidase technique at the EM level.
(Research supported by NIH and DOE).

63.16 PREPARATION OF HOMOGENEOUS POPULATIONS OF IMMATURE SCHWANN CELLS FROM EARLY EMBRYONIC SYMPATHETIC GANGLIA. D. Roufa*, C. Cornbrooks, M. Johnson and M. Bunge. Dept. Anatomy and Neurobiology, Washington University, St. Louis, MO 63110.

Studies on cellular interactions in the peripheral nervous system are greatly facilitated by the availability of tissue culture preparations containing neurons (Ns) and their associated non-neuronal cells (NNCs), Schwann cells (SCs) and fibroblasts (FBs), which can be grown as pure cell populations or in various combinations. The technique for separating functional SCs and Ns from dorsal root ganglia (DRG) was devised by Wood ('76) and relies in part on antimitotic treatment. We report here the preparation of uniform populations of SCs and associated neurites and of pure neuronal cultures from superior cervical ganglia (SCG) of early embryonic (E15) rats. SCG excised and freed of pre- and postganglionic fibers were cultured as explants in serum-embryo extract containing medium on air dried 20X diluted collagen (Bornstein, '58). NNCs proliferated rapidly (filling areas of ∿6 mm in diameter after 3d, 12 mm after 6d) and were almost exclusively in association with growing neurites. Cultures visualized by Sudan black or toluidine blue staining were composed of a uniform population of flat, cytoplasmic rich cells. Autoradiograms following ^{3}H-thymidine administration revealed a striking gradient of increased nuclear labeling from cells proximal to the explant (1-2%) to cells near the outgrowth front (99%) (in marked contrast to cultures with FBs); this gradient is consistent with results from mitogenic signal studies in DRG cultures (Salzer and Bunge, '80). Electron microscopy revealed the presence of SCs but, surprisingly, a lack of ensheathment of E15 SCG neurites, even after 20d in culture. When these NNCs were transplanted onto DRG neurites lacking SCs, however, many DRG neurites were myelinated 9d later, thus identifying these cells as SCs. Immunohistochemical staining with anti-laminin (from Timpl) resulted in patchy staining of these cells (but not neurites). No staining was obtained with human serum fibronectin antibody (from McDonald) as would be expected for FBs. These rapidly proliferating cells were very sensitive to FUDR (10^{-5}M) treatment; within a few days all NNCs could be eliminated, yielding a preparation of SCG neurites only. More extensive antimitotic treatment is required to remove NNCs from older SCG (E20) (Estridge, '77). In conclusion, the use of E15 SCG explants provides cultures of 1) neurites with a homogenous population of flat SCs with no identifiable FBs (without antimitotic treatment) and 2) neurites without NNCs using reduced antimitotic treatment. In addition, the failure of SCs to ensheathe neurites obtained from E15 SCG and grown in complete medium offers the opportunity to search for factors involved in SC differentiation. (Supported by NIH grants NS-15070, NS-09923, 5-T32-NS-07071 and GM-28002.)

63.17 CULTURES FROM ADULT MOUSE BRAIN: SURVIVAL OF ASTROCYTES AND OLIGODENDROCYTES WITH PASSAGE. S. Linderholm*, D. A. Gibson*, K. Jobe* and A. Vernadakis* (SPON: L. S. Crnic). Depts. of Pharmacology & Psychiatry, Univ. of Colo. Sch. of Med., Denver, CO 80262.

Comparisons were made of glial cell survival in brain mixed cultures derived from newborn (<1 day postnatal) and adult (>1 year postnatal) mice. Cerebral hemisphere or diencephalon was dissociated by mechanical sieving of the minced material through a 110μ pore mesh and plated on plastic petri dishes in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal calf serum (FCS). The cultures were passaged by trypsinization and replated with identical conditions as the primary culture. Survival of glial cells was evaluated using immunocytochemical markers, glial fibrillary protein (GFA) for astrocytes and glycerolphosphate dehydrogenase (GPDH) for oligodendrocytes. Fibroblastic populations were determined and quantified by immunocytochemistry, using antisera to fibronectin and the peroxidase-anti-peroxidase (PAP) method. Cellular activity was determined using biochemical assays of the astrocytic cell marker glutamine synthetase (GS) and cyclic nucleotide phosphohydrolase (CNP), a marker of oligodendrocytes. Confirmation that the cells had not become transformed in culture was supported by karyotyping of each culture type at several different passages. Immunocytochemistry conclusively confirmed that glial cells survived in cultures of adult mouse brain up to 10 passages and in newborn mouse brain cultures 5 passages up to present. In cultures from diencephalon of adult mice, the GFA-positive population remained constant with passage, but the percentage of GFA-positive cells decreased with time in culture within a passage; in cultures of newborn diencephalon, a consistent decrease in percentage of GFA-positive cells with each passage was observed. Cultures of adult and newborn cerebral hemispheres displayed a relatively constant population of GFA-positive cells with days in culture and passage. In cultures derived from newborn mouse cerebral hemispheres, both GS and CNP levels increased with passage, and also an increase in GS levels was demonstrated with days in culture; newborn diencephalon cultures displayed an increase in GS levels and a constant CNP activity with passage. In cultures derived from adult mouse, GS increased and CNP decreased in the diencephalon, whereas cerebral hemispheres displayed no change in GS and a decrease in CNP levels with days in culture. It is concluded from these data that glial cells from newborn and adult mouse can be maintained in culture through many passages. Glial cells from newborn mouse brain in culture exhibit a higher level of cellular activity as shown by GS and CNP, whereas glial cells from adult mouse brain exhibit a generalized trend of decreased cellular activity not correlated with glial cell number. (Supported by NICHD Training Grant T32 HD-07072 and a Developmental Psychobiology Research Group Endowment Fund).

63.18 THE ASTROCYTE: UNSUNG STAR OF THE CENTRAL NERVOUS SYSTEM. M.D. Noble*. Dept. Clinical Neurology, Institute Neurology, Queen Square, London WC1N 3BG, England.

Interlaced throughout the CNS are the astrocytes, thought to be the most numerous cell in the brain. Astrocytes are implicated in functions diverse as homeostatic regulation of ion fluxes and intermediary metabolism, transmitter inactivation, neuronal guidance, trophic support of neurones, and even involvement in initial stages of myelination. However, detailed evidence for any of these putative functions has been difficult to obtain.

To study details of astrocytic function, highly enriched astrocytes have been used in biochemical experiments and in reconstitution studies with neurones or oligodendrocytes. Biochemical studies have concerned peptidergic receptors (see Rougon, Noble and Mudge, this meeting). Reconstitution studies using low density platings of neurones (with Jim Cohen, Dept. Zoology, Univ. Coll. London) have demonstrated: (1) in vitro survival of CNS neurones is supported for several days by a range of cell types, but on a long term only by astrocytes, (2) neuro-glial interaction on 1-3 days after plating is unique in respect to neurite and neural soma adherence to the astrocyte, and (3) CNS neurones can be supported by killed astrocytes; living astrocytes are not required as releasers of soluble trophic factors or as partners in gap junction formation.

Low density plating of optic nerve cells (with Guy M. McKhann, Dept. Neurology, Johns Hopkins Univ.Sch.Med.) have demonstrated that astrocytes supply soluble factors required by oligodendrocytes for extended survival in vitro, and that astrocytes can profoundly influence oligodendrocyte morphology through cell-surface interactions. These studies have also shown that oligodendrocyte maturation to express the myelin-specific lipid galactocerobroside can occur in the absence of contact with any other neural cell types.

In addition, use of low density platings and cell-type specific antibodies will be discussed as an experimental strategy allowing the seperate study of neuron or oligodendrocyte populations in the absence of purified populations of these cells.

63.19 METABOLISM OF GONADAL STEROIDS BY OLIGODENDROCYTES OF CALF AND RAT BRAIN. J. Weidenfeld, J. Schorr and O. Abramsky, Labs of Exp. Endocrinology and Neuroimmunology, Dept. of Neurology, Hadassah University Hospital and Dept. of Internal Medicine, Bikur Cholim Hospital, affiliated with the Hebrew University-Hadassah Medical School, Jerusalem, Israel

It is well established that gonadal steroids can be metabolized by specific brain tissues and that these intracerebral transformations of steroids are essential for some of their CNS effects. The importance of the various CNS cell types (e.g. neurons, glia) for brain steroid metabolism is not yet clear. In the present study we investigate whether oligodendrocytes are capable of metabolizing testosterone (T) and estradiol (E_2). Oligodendrocytes were isolated from calf or male rat brains. The preparation included a trypsinization and a sucrose or percol gradient stage.

The following enzymic conversions were measured: (1) 2.5×10^6 cells were incubated with 1,2-^{3}H-T (160 pmol) in the presence of NADPH. The conversions to dihydrotestosterone were 5 pmol/hr (calf) and 4 pmol/hr (rat). This metabolite was identified by TLC chromatography and by reversed isotopic dilution. By measuring the loss of tritium to water during the incubation, it was found that approximately 3 pmol of the substrate was aromatized to E_2 in both the calf and rat cells. (2) 3×10^6 cells were incubated with either tritiated (200 pmol) E_2 or with 10 nmol of unlabeled E_2 in the presence of NADPH. The conversion of E_2 to catechol E_2 was measured by (a) formation of a tritium labeled specific phenazine derivative from tritiated 2(or 4)-hydroxy E_2 following condensation with O-phenylenediamine (Gelbke and Knuppen, Steroids 21:689, 1973) and by the radioenzymatic methylation with ^{3}H-S-adenosylmethionine in the presence of catechol-O-methyltransferase (Hoffman et al. Biochem. Pharmacol. 29:83, 1980). It was found that in both the calf and rat cells the formation of catechol E_2 was approximately 0.2 pmol/hr. These results suggest that (1) Oligodendrocytes are involved in brain metabolism of gonadal steroids and are capable of transforming these hormones to metabolites with altered biologic activity. (2) The presence of steroid metabolizing enzymes in digodendrocytes may be useful as an additional biochemical parameter for these types of CNS cells.

63.20 THE BETA-ADRENERGIC RECEPTOR SYSTEM IN HUMAN GLIOMA CELL LINES N. Shitara*, P.E.McKeever*, H. Nakamura*, T.D. Resine*, B.H. Smith and P.L.Kornblith . Surgical Neurology Branch, NINCDS, and Clinical Science Lab., NIMH, National Institutes of Health, Bethesda, MD 20205

As a study of functional characterization of human glioma cells, a mode of cellular events accompanied by beta-adrenergic stimulation was analyzed using human glioma cell lines, U-251 (Pontén, 1968) and LM (Black, 1982) and rat C6 glioma cells for comparison. ^{3}H-dihydroalprenolol binding study by Scatchard indicated different amounts of binding on each cell line (U-251: Bmax =1110 + 197 fmol/mg prot, K_D = 17.4 +3.2 nM; LM : Bmax = 655 fmol/mg prot, K_D = 14.4 nM; C6 : B max = 454 + 80 f mol/mg prot, K_D= 5.6 + 1.1 nM). L-isoproterenol (10^{-6}M) stimulation of both cell lines resulted in more than fifty times increase of c-AMP from basal activity at 15 min, and then a rapid decline at 30 min. The maximum elevation was more rapid in human lines than in C6 cells. This evidence indicates that both human glioma lines have a functioning beta-adrenergic receptor site coupling with adenylate cyclase. The receptor mediated elevation of c-AMP was regulated by both Ca+ dependent and non-dependent phosphodiesterases (PDE). Total activity of PDE started to increase at 30 min, and continued to increase to a maximum of 200% at 180 min (long term regulation).

Measurement of protein kinase activity in cytosol using γ-P^{32}ATP indicated that endogeneous cytosol protein kinase showed 158.9 ± 8.0 pmole/mg prot/min (c-AMP +), 71.6 ± 8.4 pmole/mg prot/min (c-AMP -) for exogeneous histone (50 ug) and 59.3 ± 8.2 pmoles/mg prot/min (c-AMP +), 49.3 ± 8.4 (c-AMP -), for endogenous cytosol macromolecules in U-251 cells. Comparable activities in LM were 110.9 ± 9.9 pmoles/mg prot/min (c-AMP +), 53.3 ± 6.9 (c-AMP -) for exogeneous histone, and 27.1 + 4.2 (c-AMP +) 18.4 ± 3.3 for endogeneous cytosol protein. Macromolecules in cytosol phosphorylated by endogeneous cytosol protein kinase were characterized by molecular mass on SDS-polyacrylamide electrophoresis. In U-251 the most prominent bands in the presence of c-AMP were located at 31K, 51 K and 90K. These results may provide a basis for the biological significance of beta-adrenergic regulation of human glia cells.

63.21 DEFECTS IN SPECIFIC ASSOCIATIONS BETWEEN ASTROGLIA AND NEURONS IN MICROCULTURES OF WEAVER MOUSE CEREBELLAR CELLS. M.E. Hatten, R.K.H. Liem*, M.L. Shelanski* and C.A. Mason. Dept. of Pharmacology, New York Univ. Sch. Med., New York, NY 10016.

Immunocytochemical localization of antisera raised against purified glial filament protein (AbGF), transmission electron microscopy and time-lapse video recordings were used to visualize the development of specific associations between granule neurons and astroglia in microcultures of cells dissociated from normal (+/+), heterozygous (+/wv) and weaver (wv/wv) mouse cerebella. The weaver mutation is characterized by defects in granule cell migration along Bergmann glial processes and by subsequent death of granule cells.

After 24-48h in vitro, processes of stained astroglia from normal animals (+/+) form a network which serves as a template for the organization of granule and other neurons. Time-lapse video recordings of the cultures revealed that the outgrowth of fine and broad glial processes commenced within 5-30 min of plating, followed by the specific association of neurons with astroglia (1-2h), the outgrowth of very fine neuronal processes and finally the migration of neurons on glial processes. Two types of astroglia, each with a distinct organizing influence on granule cells, were stained with AbGF; 1) cells with soma 9-10μ and either bipolar or multipolar shapes with fine, long (150μ) processes, possibly representing Bergmann glia. Neurons (1-3) were most often seen along the lengths of the arms; 2) cells having a slightly larger soma (10-12μ) and stellate shape with 4-6 processes which were slightly thicker and markedly shorter (<50μ) than type 1 astroglia. Several dozen neurons were generally nestled in between and along the arms close to the soma of these cells, possibly representing astrocytes of the internal granular layer.

In microcultures of heterozygote animals (+/wv), the number of granule cells was reduced slightly. Many stained astroglia resembled those from +/+ cerebella, but others had thickened processes and enlarged terminal "endfeet". Granule cells were found to associate with either class of stained astroglia.

Cultures from homozygous weaver (wv/wv) cerebellar contained very few, if any, granule cells and did not exhibit specific neuronal glial interactions characteristic of +/+ cells. Stained wv/wv glial cells had enlarged cell somas giving rise to swollen, stunted processes. By both immunocytochemical staining and electron microscopy, accumulation and tangling of glial filaments was seen. Supported by NIH grants NS 15429 (MEH), NS 15182 (RKHL), NS 15076 (MLS) and NS 16951 (CAM).

64.1 DOSE DEPENDENT CHANGES IN RAT LIVER PARENCHYMAL NUCLEIC ACID LEVELS ASSOCIATED WITH ACUTE SOMAN TOXICATION, A. Anthony*, J. Doebler*, T. Bocan*, R. Moore* and T.-M. Shih (SPON: J.F. Glenn). Penn State Univ., Univ. Park, PA 16802 and US Army Med. Res. Inst. of Chem. Def., APG, MD 21010

Cytophotometric analyses were made of RNA, DNA and protein changes in hepatocytes from male Sprague-Dawley x Wister rats treated A.M. or P.M. with soman (65, 120 or 195 µg/kg, s.c.) to characterize alterations in liver nucleic acid metabolism associated with anticholinesterase poisoning. Plasma and erythrocyte cholinesterase was also assayed to monitor extent of enzyme inhibition. A dose-dependent suppression of hepatocyte RNA content was evidenced in both A.M. and P.M. injected rats, with RNA depletion being more severe in P.M. animals. Significant elevations in Feulgen-DNA yield were evidenced in P.M.-injected, but not in A.M. injected rats, indicating that soman can elicit an alteration in the physicochemical state of the chromatin. Slight A.M.-P.M. differences were also evidenced in total hepatocyte protein content of soman toxicated rats. Thus, the overall findings indicate that soman effects a marked alteration in hepatocyte transcriptional-translational capability and that diurnal fluctuations exist with respect to hepatocyte responsiveness to anticholinesterase poisoning. These observations also support the premise that the nature and severity of soman-induced impairment in regulatory aspects of nucleic acid metabolism in cholinergic tissue compartments is dependent on the functional state of individual cell populations at the time of poisoning. (Supported in part by USAMRDC Grant DAMD 17-81-C-1202).

64.2 ISOLATION AND CHARACTERIZATION OF SYNAPTIC PLASMA MEMBRANE (SPM) MARKERS: A 240K ANTIGEN AND ITS 58K SUBUNIT. A. Korenovsky*, V. Ciccarone*, S.P. Mahadik, and M.M Rapport. Div. of Neuroscience, NY State Psychiatric Inst. and Dept. of Biochem., Coll. Phys. & Surg., Columbia U., N.Y., N.Y., 10032.

Few SPM proteins have been isolated and characterized. Using rabbit antisera to a purified SPM fraction from rat brain, five antigens can be detected and quantified with primary localization in SPM (content in subcellular fractions). One, a 58K antigen, showed the following developmental patterns in cortex and cerebellum. It was detected at E14, remained low to P9, increased rapidly to P28, fell to about half its maximal level at P60, and then slowly increased to a high value at P180. In cerebellum, 58K appeared at E14, increased rapidly at birth, decreased markedly at P5, increased to a maximal value at P14, and then slowly decreased, disappearing at P180. Since structure may provide clues to the role of 58K in developmental processes, we have purified this antigen in its oligomeric form. The following procedure was monitored by rocket immunoelectrophoresis. The SPM from cortex was extracted with 5 vol of chloroform-methanol (2:1) followed by repeated extractions with theoretical upper phase (KCl). The antigen in the upper phases was purified by isoelectric precipitation and was obtained pure by filtration on a column of Bio-gel A0.5M. The antigen gave a single band on SDS-gradient PAGE in the presence of mercaptoethanol, with estimated Mr of 58K. The yield was considerably improved by starting with the total membrane fraction. 58K antigens obtained from SPM and total membrane fractions were indistinguishable by 2-D gel electrophoresis. The size of the isolated antigen was 240K by gel filtration analysis and 58K by SDS-gel electrophoresis suggesting four subunits of the same size. The isoelectric point was 4.2. The pure antigen was stable for several months. The partially purified antigen was degraded and lost immunochemical activity rapidly at 37C in the presence of Ca indicating it to be proteinaceous. Degradation and loss of activity also followed treatment with trypsin or chymotrypsin but not with nucleases. The ratio of absorbance at 280 nm to 260 nm was 1.10. On SDS-slab gels stained with Coomassie Blue the antigen gave an intense pink color (similar to glycoproteins) but did not stain with Schiff's reagent after periodate treatment. Neuraminidase caused no loss of activity suggesting the absence of neuraminic acid. Thus, a membrane protein (synaptic marker antigen) has been isolated in a pure oligomeric form. It remains in aqueous solution without aid of detergents, is very acidic, and should permit the raising of monospecific antisera to study histological localization and biological properties.

64.3 BODIAN SILVER STAIN AND AIF ANTIBODY BIND TO DIFFERENT REGIONS OF MYXICOLA NEUROFILAMENT PROTEIN, L.L. Phillips, R.J. Lasek, L. Autilio-Gambetti* and P. Gambetti. Dept. of Anatomy and Institute of Pathology, Case-Western Reserve Univ. Sch. Med., Cleveland, OH 44106.

The application of the Bodian silver method to electrophoresed neural proteins has shown that silver selectively stains neurofilament molecules (NFs) in a wide variety of invertebrate and vertebrate species (Phillips, L., et al., Anat. Rec., 202: 149A, 1982). Recent immunobinding studies have revealed a monoclonal antibody (AIF) which binds to a common site on all intermediate filament (IF) types (Pruss, R., et al., Cell, 27: 419, 1981). We report the results of studies designed to separate and localize the binding of these two markers within the NF molecule. Myxicola NFs were chosen for this study because an endogenous Ca^{++} activated protease selectively cleaves the protein into a head and tail region (Eagles, P., et al., Biochem. J., 199: 101, 1981).

Axoplasm was isolated, homogenized and exposed to 10 mM $CaCl_2$ for one hour. EGTA was then added to terminate the enzyme activity and samples were centrifuged, separating a soluble fraction which contained the head region of the NFs and an insoluble fraction which contained the NF tail regions. Polypeptides from both fractions were analyzed by 1D SDS-PAGE and stained with Coomassie blue. The gels were subsequently stained either by the modified Bodian method or the PAP method, using AIF.

The major cleavage products present in the soluble fraction (94, 70-80, 65-68 k daltons) and insoluble fraction (55, 57 k daltons) were similar in molecular weight to those previously identified by Eagles (Ibid., 1981). The major Coomassie stained bands present in the soluble fraction (94, 70-80, 65-68 k daltons) bound Bodian silver, but not AIF. In the sedimented fraction the major insoluble fragments (55,57 k daltons) stained intensely with AIF, but did not bind silver.

We conclude that the binding sites for a NF specific marker (Bodian stain) and a general IF marker (AIF antibody) are located in different domains of Myxicola NFs. The association of the general IF domain with the sedimented fraction and the specific NF domain with the released fraction suggests that NFs conatin a backbone which has some homology with other IF proteins and that the NF specific domain is cleaved from the backbone by the endogenous Ca^{++} activated protease.

64.4 NOREPINEPHRINE STIMULATED PHOSPHORYLATION OF GLIAL FIBRILLARY ACIDIC PROTEIN (GFA). Edward T. Browning and Monica Ruina*. Dept. Pharmacol., Rutgers Medical School, Piscataway, N.J. 08854.

GFA forms intermediate diameter filaments in the cytoskeleton of astrocytes. Past studies demonstrated that vimentin, another intermediate filament protein which occurs in glial cells undergoes norepinephrine stimulated phosphorylation in C-6 glioma cells (Browning and Sanders, J. Cell Biol., 90, 803). Present studies show that this is true of GFA as well. Authentic GFA was prepared from rat spinal cord as a cytoskeletal fraction and resolved by gradient slab gel electrophoresis and 2-dimensional (2-D) gel electrophoresis. GFA migrated in 2-D gels as 3 charge variants, above the alkaline edge of actin. This migration was very similar to that of a previously described phosphoprotein (50K-6.1) which is 3.3-fold increased in ^{32}P-content by norepinephrine in C-6 glioma cells (Groppi & Browning, Mol. Pharmacol., 18, 427). Coelectrophoresis of the GFA preparation and ^{32}P-labeled C-6 whole cell extract showed the ^{32}P-50K-6.1 spot to lie in line with GFA in size but slightly to the acidic side typical of the phosphorylated derivative of a protein. GFA has been reported to be present in C-6 cells at low concentration but is increased in abundance following dibutyryl cyclic AMP (dbcAMP) treatment. The endogenous stained protein associated with ^{32}P-50K-6.1 was inducible by dbcAMP. The norepinephrine stimulation of putative GFA phosphorylation was reproduced in dbcAMP treated cells. Peptide mapping of the respective proteins was used to test the hypothesis that GFA was indeed identical with 50K-6.1. For mapping dbcAMP induced cultures were labeled with either ^{32}Pi or ^{35}S-methionine and whole cell extracts were resolved by 2-D gel electrophoresis. The ^{35}S-labeled putative GFA, its acidic satellite, and ^{32}P-50K-6.1 were cut from dry gels and mixed with sufficient authentic GFA for Coomassie staining of GFA peptides. Mapping was performed according to Cleveland et al. (J. Biol. Chem., 252, 1102) and autoradiographic peptides were compared to stained GFA peptides. Both the ^{35}S-putative GFA and its acidic satellite gave rise to peptides which comigrated 1 for 1 with those for authentic GFA. The ^{32}P-labeled protein gave rise to peptides that formed a subset of the stained GFA peptides typical of a protein labeled selectively at one or a few positions within the primary structure. The conclusion was drawn that protein 50K-6.1 is identical to a phosphorylated form of GFA. Therefore, GFA undergoes norepinephrine stimulated phosphorylation in C-6 glioma cells. (Supported by NSF Grant BNS 81-10564.)

64.5 A TWO DIMENSIONAL GEL ANALYSIS OF PROTEINS OF NEURONAL AND NEUROGLIAL NUCLEI FROM RAT BRAIN. M. R. Wells and J. J. Bernstein. Lab. of Central Nervous System Injury and Regeneration Research, Veterans Administration Medical Center, and Dept. of Physiology, George Washington University, Washington, D. C. 20422.

The proteins of neuronal and neuroglial nuclei from adult rat brain were examined by adaptation of standard two dimensional gel electrophoresis methods (isoelectric focusing and molecular weight). An ultrasensitive silver stain was used for detection of proteins (Oakley, et al., Analyt. Biochem. 105:361, 1980). Neuronal and neuroglial nuclei were separated from either whole brain or cerebral cortex by ultracentrifugation methods (Stoykova, et al., J. Neurochem 33:931, 1979). In some instances nuclei were washed in 0.2% Triton to remove possible contaminants. Samples of nuclei were solubilized and incorporated into isoelectric focusing gels (Jackowski, et al., Can. J. Biochem. 54:9, 1976) containing broad range ampholines (pH 3-11). Focused proteins (3000 volt hours) were separated in the molecular weight dimension of 10% polyacrylamide SDS gels. A 20-30 µg protein sample could be separated into over 150 proteins. The proteins of neuronal and neuroglial nuclei were similar with focusing points extending over a broad range (pH 5-10) and an apparent Mr range of 20,000-130,000 Daltons. The nuclear proteins characteristically contained several groups of proteins of differing isoelectric point but of the same molecular weight. The groups were distributed over most of the electrofusing and molecular weight ranges. Analysis of subtractions of nucleoli from neurons and neuroglia indicated that there are 15-20 primary proteins associated with nucleoli. These are located in the acidic range and have an apparent Mr of 30,000-70,000. Experiments to date indicate that there are some differences in the nucleolar proteins from neurons and neuroglia. More study will be necessary to rule out the possibility of contaminants. The methods employed in these experiments may be useful for the study of nuclear proteins in more localized brain regions than has been previously possible. Supported by the Veterans Administration and Grant No. NS-16979 from the National Institutes of Health (NINCDS).

64.6 MONOCLONAL ANTIBODIES TO SPECIFIC NEURONAL AND GLIAL MOLECULES A.L. De Blas and R.O. Kuljis. Dept. of Neurobiology and Behavior, S.U.N.Y. at Stony Brook, N.Y. 11794.

Monoclonal antibodies to a synaptosomal membrane fraction from rat cerebral cortex were obtained. Spleen cells from immunized BALB/c mice were fused with the mouse myeloma line P3X63Ag8. Antibodies produced by the hybridoma cells that were directed to molecules in the synaptosomal membrane fraction were detected by an indirect solid phase radioimmunoassay. Fifty-three hybridoma lines secreted antibodies that bound to antigens present in the synaptosomal membrane fraction. The cellular and topographic distribution of the corresponding antigens was studied immunocytochemically by the peroxidase-antiperoxidase and fluorescence methods. Intense staining could be obtained with eight of the monoclonal antibodies, while no staining could be obtained with the culture medium of the parental myeloma line. Five of the antibodies demonstrated specificity for neurons. Two monoclonal antibodies bound specifically to both astrocytes and Bergmann glia. Another antibody recognized neurons preferentially, but some astrocytes were also stained. The five neuron-specific monoclonal antibodies displayed different staining patterns suggesting that each recognizes a different molecule. The two monoclonal antibodies specific for both astrocytes and Bergmann glia presented a staining pattern identical to that obtained by a conventional antiserum to glial fibrillary acidic protein (GFA) which was kindly supplied by Drs. D. Dahl and A. Bignami. Furthermore, these two latter monoclonal antibodies did not stain either the blood vessel walls or the leptomeninges, a fact suggesting that GFA and not vimentin (also found in astrocytes and Bergmann glia) is the molecule recognized by these two antibodies. Additional work is needed for the complete characterization of these specific neuronal and glial molecules.

64.7 PRODUCTION AND CHARACTERIZATION OF A MONOCLONAL ANTIBODY TO S-100. B.D. Boss, E.A. Haan* and W.M. Cowan. The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

S-100 is a highly acidic, calcium-binding protein which is said to be localized either solely within the cytoplasm of glial cells or in glial cells and neuronal nuclei. To date, only polyclonal antisera to this protein have been available for immunohistochemical and quantitative analyses. In order to clarify the cellular localization of S-100, we have developed a monoclonal antibody to this protein. This monoclonal antibody reacts only with glial cells in brain sections and in tissue culture. The antibody was produced in a fusion of Sp2/0 15-4 Ag5oua mouse myeloma cells and spleen cells from a female Balb/c mouse immunized with bovine S-100 (kindly provided by Dr. Blake W. Moore, Washington University, St. Louis). Hybridoma supernatants were screened for reactivity to S-100 in an enzyme-linked immunosorbant assay. Cloning was accomplished by limiting dilution. Ascites tumors were produced in normal, pristane-treated Balb/c mice. The immunoglobulin subclass of the antibody was determined to be IgG_1 in a typical Ouchterlony immunodiffusion experiment using 10-fold concentrated culture supernatant.

To demonstrate that the monoclonal antibody was indeed reacting with the major proteins in our S-100 preparation and not to a minor contaminant, S-100 was separated into two bands by electrophoresis on cellulose acetate. These protein bands were subsequently Western blotted onto nitrocellulose and reacted with the antibody. Bound antibody was visualized using an indirect immunoperoxidase method. It was found that the monoclonal antibody reacted with both protein bands present in our S-100 preparation.

Cellular localization of the antibody binding sites has involved indirect immunoperoxidase and immunofluorescence staining of fixed adult rat brain sections and of neonatal rat dentate gyrus cell cultures. To identify the cell types labeled in our in vitro system, we conducted double labeling experiments utilizing tetanus toxin and glial fibrillary acidic protein as neuron- and astrocyte-specific markers, respectively. It was found that, in both the brain sections and cell cultures, astrocytes were clearly labeled by this monoclonal antibody. There was no labeling of neuronal cells in either system. Ongoing studies with this antibody are pursuing the developmental expression of S-100.

Supported by NIH grants NS-16980 and EY-01255, and by the Clayton Foundation for Research-California Division.

64.8 COMPARISON OF PROTEINS SYNTHESIZED BY ASTROCYTES AND NEURONS IN PRIMARY CULTURES. F. P. White and L. Hertz*. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 3V6; and Department of Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0.

We have previously characterized the proteins synthesized by astrocyte enriched primary cultures (AEPC) using SDS-PAGE and found alterations in the polypeptides synthesized by AEPC as a function of both their age in vitro and the presence in the culture medium of dibutyryl cyclic AMP which alters their morphology (Neurochem. Res. 6: 353-364). We have recently extended these observations by characterizing the protein synthesized by both AEPC and neuron enriched primary cultures (NEPC) using two dimensional electrophoresis.

APEC and NEPC were prepared from Swiss albino mice cerebral cortex. Cultures were maintained for various times in vitro and some AEPC were treated with dibutyryl cyclic AMP after 2 weeks in vitro. Proteins synthesized by the cells in culture were labelled for 1 hour with ^{3}H-leucine in a MEM which contained 1/800 of the normal amino acid supplement. Proteins from each culture were extracted and separated first by isoelectric focusing on a pH gradient from 7.0 to 3.5, and then by SDS-PAGE on a 7 1/2% polyacrylamide gel. Radioactive proteins were visualized by autofluorography. Polypeptides with molecular weights between 250 and 27 kilodaltons having isoelectric points between 7.0 and 3.5 which are soluble in 8 M urea and 10% NP-40 were resolved into single species by this procedure.

The rate of protein synthesis in both AEPC and NEPC when labelled in the presence of the normal concentration of amino acids was approximately the same, 7.0 nmol/hr/mg protein. AEPC and NEPC synthesized many of the same proteins. Some of these were among the 10 most abundant species synthesized by each type of culture and included α, β, and γ actin, 73K 5.6 (a protein related to stress proteins), glucose regulated protein, 88K 5.4, and 45K 5.7 (creatine kinase). Major proteins synthesized by NEPC but not AEPC included α and β tubulin. AEPC synthesized about 8 major proteins which were not found in NEPC. These included 62K 5.0, 59K 5.1, 45K 5.5, 38K 5.2, and a group of proteins with molecular weights between 36 and 38 kilodaltons and isoelectric points around 5.7. The synthesis of all these AEPC proteins were altered by dibutyryl cyclic AMP.

These results should help in determining the cellular origin of proteins synthesized by the brain in vivo both during development and in response to various stresses. (Funded by MRC Canada).

64.9 CHARACTERIZATION OF A VERY-RAPIDLY-TURNING-OVER CYCLIC-AMP BINDING PROTEIN IN NEUROBLASTOMA CELLS. J. M. Gilbert, V.S. Sapirstein and P. Strocchi*. Mailman Res. Ctr., McLean Hosp., Eunice Kennedy Shriver Ctr. and Harvard Medical School, Boston, MA 02115

Pulse-chase experiments with [^{35}S]-L-methionine were carried out with neuroblastoma 2A and NIE-115 cells grown on monolayers in order to detect rapidly-turning-over proteins. Radiolabel was added for 15 min in order to detect rapidly synthesized proteins. The cells were broken by Dounce homogenization and after removal of nuclei, the soluble and particulate proteins were separated by ultracentrifugation and then analyzed by two-dimensional gel electrophoresis (2DGE) followed by fluorography. In order to detect those proteins which were rapidly catabolized, a 15 and 30 min chase with cold L-methionine was carried out, followed by analysis of radiolabeled proteins by 2DGE and fluorography. Among all the proteins labeled during the 15 min incubation with [^{35}S]-L-methionine, three proteins had almost completely disappeared after the 30 min chase - 54K 5.5, 57K 5.8 and 84K 5.8. While the specific activity of the 57K 5.8 and 84K 5.8 proteins had diminished after 15 minutes, the 54K 5.5 was almost completely catabolized after the 15 min chase with cold L-methionine. Thus, we would estimate the half lives of the 84K 5.8 and 57K 5.8 proteins to be between 10 and 15 min and the half life of the 54K 5.5 protein to be 5 min or less. In additional studies we purified radiolabeled proteins (after the 15 min pulse) by cyclic-AMP affinity chromatography followed by 2DGE and fluorography. The 54K 5.5 protein bound to cyclic-AMP along with other proteins at both 54K and 48K, which are in the RI and RII classes of cyclic-AMP binding proteins, respectively. Both RI and RII proteins are rapidly synthesized during the 15 min pulse but only the 54K 5.5 cyclic-AMP binding protein disappeared after a 15 min chase. In order to rule out the possibility that the 54K 5.5 is removed from the soluble pool into a particulate fraction, we analyzed the particulate components during the pulse-chase experiments; movement of the 54K 5.5 protein into membranes during the chase did not occur. Also, during the chase we did not detect the appearance of a new radiolabeled protein in the 2D electrophoretograms. In summary, we have identified in two neuroblastoma cell lines three soluble proteins, which, by virtue of their rapid turnover, could act as regulators of acute changes in neural function. One of these proteins, 54K 5.5, is within the RII class of cyclic-AMP binding proteins and has an estimated half-life of 5 min or less. Theoretically, significant changes in the cellular concentration of the 54K 5.5 RII can be affected within seconds by changes in its rate of synthesis and/or catabolism. Therefore, we postulate that the 54K 5.5 protein may function in regulating rapid changes in cyclic-AMP mediated processes in the CNS. Supported by NIH grants MH70713, MH362204, AG02126, HD05515 and NS16186.

64.10 STUDIES ON GLIAL GROWTH FACTOR. G.E. Lemke and J.P. Brockes. Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

We have previously reported on the properties of glial growth factor (GGF), a novel protein mitogen, present in the brain and pituitary, which triggers DNA synthesis and cell division in cultured rat Schwann cells, astrocytes, and fibroblasts (Brockes,J.P. Lemke, G.E., and Balzer, D.R., J. Biol. Chem., 255:8374,1980).Our biochemical characterization of this molecule has progressed along the following lines:

(1) We have developed methods for recovering GGF activity following SDS-polyacrylamide gel electrophoresis. These methods have allowed us to demonstrate that growth factor activity resides in a molecule of molecular weight 31,000, and to identify this protein unambiguously.

(2) By using immunoautoradiographic methods in conjunction with a panel of monoclonal antibodies to GGF (Lemke,G.E. and Brockes, J.P., In: Monoclonal Antibodies to Neural Antigens, Cold Spring Harbor Press: 133, 1981), we have shown that all antibodies which specifically precipitate GGF activity also bind exclusively to the 31K protein.

(3) We have investigated the possible relation of GGF to the platelet-derived growth factor (PDGF), a potent fibroblast mitogen isolated from platelet lysates. Like PDGF, GGF is a very basic protein of molecular weight 31,000 whose activity is destroyed by reducing agents, but which is relatively resistant to high temperature and low pH. While we have found that purified PDGF (a gift from Dr. R. Ross, Univ. of Washington, Seattle) shows little mitogenic activity against Schwann cells, we hypothesize that both proteins are members of the same family of growth factors, some of whose target cells (e.g., fibroblasts) overlap.

(4) In order to permit further biochemical characterization of GGF, we have recently purified the molecule from 20,000 bovine anterior lobes by a combination of the biochemical methods previously described followed by SDS gel electrophoresis.

64.11 PROTEIN SULFATION ON TYROSINE RESIDUES IN INTACT AND LYSED PC12 CELLS. Raymond Lee+ and Wieland Huttner. Max-Planck-Institute for Psychiatry, Dept. of Neurochemistry, 8033 Martinsried, West-Germany.

Protein sulfation on tyrosine residues has recently been shown to be a wide-spread protein modification. We have now studied tyrosine sulfation of specific proteins in PC12 rat pheochromocytoma cells. PC12 cells, grown in the absence of nerve growth factor, were labeled in vivo by incubation with inorganic (^{35}S)-sulfate. Cellular proteins were then separated by one- or two-dimensional polyacrylamide gel electrophoresis followed by autoradiography of the gels. Analysis of individual proteins for radioactive tyrosine-O-sulfate indicated that the major tyrosine-O-sulfated proteins were two pairs of acidic polypeptides, designated according to their apparent molecular weights in kilodaltons as p113, p105, p86 and p84. When PC12 cells had been labeled in vivo by incubation with inorganic (^{32}P)-phosphate, four of the major cellular phosphoproteins showed electrophoretic mobilities in two-dimensional gels that were identical to those of the four tyrosine-O-sulfated p113, p105, p86 and p84. These four phosphoproteins contained radioactive phosphoserine. Peptide mapping after limited proteolysis of either (^{35}S)-sulfate labeled or (^{32}P)-phosphate labeled proteins provided evidence that the four phosphoproteins and the four tyrosine-O-sulfated proteins were identical. These peptide maps also revealed a high degree of sequence homology between p113 and p105, and between p86 and p84. Exposure of PC12 cells to nerve growth factor altered the relative amounts of tyrosine-O-sulfated p113 and p105. Tyrosine-O-sulfated p113, p105, p86 and p84 were obtained in the soluble fraction prepared from in vivo labeled PC12 cells. Furthermore, these four proteins were found to remain soluble after 5 min at 100°C.

Protein sulfation was also studied in hypoosmotically lysed PC12 cells. After incubation of PC12 cell lysates with (^{35}S)-3'-phosphoadenosine 5'-phosphosulfate (PAPS), the proteins were found to contain radioactive sulfate on tyrosine residues. This observation suggests, in PC12 cells, the presence of a specific tyrosylprotein sulfotransferase that uses PAPS as a sulfate donor.

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64.12 EXISTENCE OF 2-DEOXY-2,3-DEHYDRO-N-ACETYL NEURAMINIC ACID IN RAT AND BOVINE BRAINS. M. Saito* and A. Rosenberg* (SPON: I.R. HELD). Dept. of Biochem. and Biophys. Loyola Univ. Stritch School of Medicine, Maywood, IL 60153.

The sialic acid derivative, 2-deoxy-2,3-dehydro-N-acetyl neuraminic acid (2,3-dehyro NeuAc) has been found as a natural compound in the urine of a sialuria patient (J.P. Kamerling et al. Eur. J. Biochem. 56,258 (1975)) and discovered to be a regular constituent in urine, serum and saliva from nomal humans (J. Haverkamp et al. Hoppe-Seyler's Physiol. Chem. 357 S. 1699 (1976)). The biological significance of this compound is still unclear although its strong inhibitory activities on sialidases has been reported (P. Meindl et al. Hoppe-Seyler's Physiol. Chem. 350, S. 1088 (1969)). We found this compound in the extracts from rat and bovine brains. The 2,3-dehydro NeuAc was eluted from Dowex1 columns in the same fractions as N-acetyl-neuraminic acid (NeuAc) and could be separated by cellulose column chromatography from NeuAc. The materials from cellulose chromatography were identified by thin layer chromatography (TLC) and gas-liquid chromatography with authentic reference compounds. Since it has been reported that CMP-NeuAc can be degraded in vitro to 2,3-dehydro NeuAc (J.M Beau et al. abstr. VIII Journees sur la Chimie et la Biochime des Glucides, Chamerolles, France), the possibility should be investigated that 2,3-dehydro NeuAc was formed during the isolation procedure.

For this purpose, rats were anesthetized with ether and injected intracerebrally with ^{14}c-N-acetyl-D-mannosamine for labeling NeuAc metabolically. Three hours and 40 hours after the injection, rats were decapitated after being anesthetized.

The radioactive materials were extracted using the same procedure as before. The NeuAc fractions from Dowex-1 column chromatography were analyzed on TLC. The sample from rats killed 3 hours after the injection had no radioactivity in the area where carrier 2,3-dehydro NeuAc migrated, in contrast, the sample from rats killed after 40 hours showed radioactivity in that area. The radioactivity was 8% of that on the area where NeuAc migrated. As already reported (W. Ferwerda et al. J. Neurochem. 36, 1492-1499 (1981)) the specific activity of radioactive CMP-NeuAc is much higher at 3 hours after the injection of labelled Man-NAc than 40 hours (more than ten times). If 2,3-dehydro NeuAc was formed from CMP-NeuAc during isolation procedure, much more radioactivity can be expected in 3 hr. labeling rat brain than in 40 hr. labeling rat brain. The results suggest strongly that 2,3-dehydro NeuAc is a naturally formed metabolite in brain.

64.13 IMMUNOCYTOCHEMICAL LOCALIZATION OF GM_1-GANGLIOSIDE IN PRIMARY CULTURES OF BRAIN CELLS FROM NEWBORN RATS. Hiroaki Asou* and Eric G. Brunngraber. Neurochemistry Research Unit, Missouri Institute of Psychiatry, Department of Biochemistry, University of Missouri-Columbia, 5400 Arsenal St., St. Louis, MO 63139.

A procedure for cultivation of neonatal brain cells was developed. Neurons, oligodendrocytes and astrocytes showed morpholigical differences, but these cells were more clearly identified by the use of antiserum to cell-specific constituents. Oligodendroglia and astroglia were repsectively identified by antisera to cerebroside and glial fibrillary acidic protein. Antiserum to ganglioside GM_1 stained neurons and oligodendroglia; astroglia were not stained. Using histochemical methods, hyaluronectin a hyaluronic acid binding glycoprotein, was found to be localized in the oligodendroglia.

64.14 EFFECT OF TEMPERATURE - AND OXYGEN - ACCLIMATION ON PHOSPHOLIPIDS OF GOLDFISH BRAIN MICROSOMES AND MITOCHONDRIA. Michael Chang* and Betty I. Roots. Dept. of Zoology, Univ. of Toronto, Erindale College, Mississauga, Ontario, L5L 1C6, Canada.

The effect of temperature and oxygen acclimation on phospholipids of mitochondria and microsomes isolated from goldfish, Carassius auratus, brain was investigated. All fish were temperature acclimated at 25°C and 5°C whereafter some were subjected to varying levels of oxygen tension.

In both membrane fractions, phospholipid composition was altered when the acclimation temperature was changed (25°C-5°C). While the proportion of ethanolamine- to choline- glycerophosphatide (GPE/GPC) increased significantly at 5°C, a decrease in the P-GPE (phosphatidal ethanolamine) in relation to GPE (total ethanolamine glycerophosphatides) also resulted.

Oxygen levels to which fish were acclimated were hypoxia (OXY I), normoxia (OXY II) and hyperoxia (OXY III). Irrespective of temperature (25°C or 5°C), both membrane fractions exhibited a replacement of GPC with GPE when the oxygen tension increased; the GPE/GPC ratio decreased at the hypoxic level. Also, an elevation of the P-GPE:GPE ratio was observed when the concentration of oxygen was raised. Thus, the effect of oxygen tension to some extent antagonises the change in the P-GPE:GPE ratio observed with temperature acclimation.

Significant effects of acclimation temperature on acyl group composition of the phospholipid classes were also apparent. The changes were essentially similar for both fractions. For the ethanolamine classes, phosphatidyl choline and REST (serine and inositol phosphoglycerides) the most striking change experienced with cold acclimation is the increase in the n-6 fatty acids with concomitant decreases in the n-3 family. This was best reflected in the n-6/n-3 ratio. Irrespective of temperature (25°C or 5°C), the same trend was evident when oxygen concentration was raised (OXY II-OXY III at 25°C and OXY III at 5°C). Thus, oxygen effects enhance the changes occurring with temperature acclimation.

64.15 VITAMIN E DEFICIENCY EXACERBATES MEMBRANE DEGRADATION IN COMPRESSION-INDUCED BRAIN SWELLING. S. Yoshida*,[1] R. Busto*[1], M.D. Ginsberg*[1], K. Abe*[1,2], M. Santiso*[1], O. Alonso*[1], and P. Scheinberg*[1]. [1]Cerebral Vascular Disease Research Center, Dept. of Neurology, Univ. of Miami School of Medicine, Miami, FL. 33101, [2]Eisai Research La., Ibaraki, Japan.

Compression-induced brain swelling can be ameliorated by prior supplementation of vitamin E (VE) (Neurology 1982; 32(2):A109). This brain swelling is characterized by increases of brain water and sodium contents, little change in potassium and is associated with leakage of serum protein. To investigate the mechanisms of VE protection in vasogenic edema, the total fatty acid composition of the brain was studied in the same model. Three groups of rats raised on VE-deficient (-D), -normal (-N) and -supplemented (-S) diets for 8 - 10 weeks were subjected to a 24 hour-period of focal brain compression produced by insertion of a Silastic plug into the right epidural space. At 24 hours after plug removal, the brains were frozen in situ. Shamp-operated animals in each dietary group served as controls.

Plasma VE level assayed by the method previously reported (Neurosci Abs 1981; 7:912) was 0.6 (mean) μmol/l in the VE-D group, 9.7 in the VE-N and 28.0 in the VE-S. Four - five mg of grounded brain tissue was dehydrated by 2,2' dimethoxypropane. Following evaporation, anhydrous HCl-methanol and heneicosanoic acid, an internal standard, were added and the sealed test tubes were heated for 2 hours in a water bath at 82-84°C to complete transmethylation. Fatty acid methylesters were extracted with hexane-diethyl ether (20:1, v:v) and analyzed by gas-liquid chromatography. In control brains of VE-D rats, the levels of palmitic (C16:0), stearic (C18:0), oleic (C18:1), arachidonic (C20:4) and docosahexaenoic (C22:6) acids were 120 (mean), 121, 127, 51 and 66 m mol/kg dry weight respectively and did not differ among the different dietary groups. Following compression, brain fatty acids were decreased ipsilaterally in VE-D rats ($p<0.05$); the levels were 93 (C16:0), 88 (C18:0), 93 (C18:1), 41 (C20:4) and 49 (C22:6) m mol/kg dry weight. Fatty acid levels ipsilateral to compression in VE-N and -S rats were not siginificantly different from those of corresponding controls.

The results suggest that VE prevents biomembranes from degradation in edematous brain. Saturated and mono- and poly-unsaturated fatty acids were decreased to a similar degree in brain-compressed VE-D rats. The results support the action of VE as a membrane stabilizer rather than as a free-radical chain-breaker.

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64.16 STAGE SPECIFIC ANTIGENS O5 TO O11 ON OLIGODENDROCYTE CELL SURFACES DETECTED BY MONOCLONAL ANTIBODIES. I. Sommer, C. Lagenaur and M. Schachner, Dept. of Neurobiology, University of Heidelberg, Heidelberg, Fed. Rep. Germany.

The developmental expression of seven oligodendrocyte-specific cell surface antigens designated O5 to O11 was investigated by indirect immunocytological methods in fresh frozen sections and monolayer cultures of normal and myelin deficient jimpy mutant mice. Antibodies were obtained from hybridoma clones as described previously (Sommer and Schachner, Develop. Biol., 83:311 (1981). In sections of adult mouse brain all antibodies reacted only with white matter tracts. Cell-surface and cell-type specificity for oligodendrocytes was confirmed in monolayer cultures of viable early postnatal cerebellar cells.

The detectability of O antigens was age-dependent. In sections of spinal cord antigens O5,O6 and O7 were detectable at birth followed by O8 at day 2, by O9 and O10 at day 4 and O11 at approximately day 7. In sections of 7-day-old cerebellum O5,.O6, and O7 were detected in all areas of presumptive white matter tracts. Detectability of O10 and O11 was confined to the peduncular region near the fourth ventricle. O8 and O9 were found in presumptive white matter tracts extending beyond those of the peduncles, but not encompassing the more extensive expression of O5, O6 and O7. In monolayer cultures of 7-day-old mouse cerebellum maintained for three days in vitro, O5 and O6 were found on approximately 90% of all O4 positive oligodendrocytes. This percentage was highest for O5 and O6 and decreased for the other antigens in the following order: O7, O8, O9, O10 and O11. O11 was detectable on less than 5% of all O4 positive cells.

In sections of spinal cord from Jimpy mice, detectability of antigens O5 to O9 was delayed by several days as compared to normal control littermates. O5 and O6 were detectable at day 3 (the earliest stage tested) but in much lower amounts than in control littermates. O7 was detected at day 5, O8 and O9 at day 13. O10 and O11 could not be detected within the range of ages tested (day 3 to day 32). Antigens O5 to O10 were also found in human white matter.

We conclude that antigens O5 to O11 define different stages in the maturation of oligodendrocytes and myelin.

64.17 REGIONAL VARIATION OF AXON CALIBER, FIBER THICKNESS AND GLIAL CELLS ALONG THE OPTIC NERVE OF THE ADULT RAT. R. P. Skoff and K. M. Liu*. Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

In previous studies (Skoff, Neurosci. Lett., 7:191 1978; Skoff et al., J. Comp. Neurol., 191:237, 1980), we showed that myelination along the rat optic system begins at several different sites. At these sites, the diameter of the axon and the thickness of the myelin sheath is greatest. In addition to fiber heterogeneity, the number of glial cells shows many peaks and troughs along the nerve. The variability of these optic nerve components raises questions not only about the factors causing it but also about its persistence into adulthood. The present study investigates the caliber of the axon and myelin sheath as well as the distribution of neuroglia along the adult rat optic nerve.

After perfusion with aldehydes, the optic nerve, chiasm and tract of six Charles River strain adult rats were embedded in Araldite using routine electron microscopic procedures. The entire nerve was sectioned transversely at closely spaced intervals (200 um). The number of neuroglial cells, the area of the axons and of their myelin sheaths were quantitated at many sites along the nerve. The perimeter of axons and their surrounding sheaths were traced with a HIPAD Digitizer interfaced with an Apple computer. The data were analyzed using Bioquant software.

The area and diameter of all fibers increases 30% to more than 100% from behind the lamina cribrosa to the chiasm. The largest fibers increase the most, usually doubling in area and diameter. The area of the largest axons also doubles from the eye to the chiasm. In the orbit, the average area of fibers is extremely variable from animal to animal but this spread decreases towards the chiasm where the variation is less than 15% from rat to rat. The area and thickness of the myelin sheath increases rostro-caudally but the largest change occurs in the orbit. The number of glial cells is highest right behind the lamina cribrosa, decreasing sharply in the orbit and then remaining relatively stable.

The results show that the regional variation observed in the adult optic nerve is quite different from that of the developing nerve. These observations suggest that (1) local factors contribute to the regulation of fiber thickness, (2) the rate of myelination may be different at different sites along the nerve and (3) the effect of these local factors may change with time. (Supported by NIH NS 15338).

64.18 REAL-TIME FLUORESCENCE POLARIZATION ASSAY FOR PHOSPHOLIPASE A_2. J.A. Monti, M.R. McBride*, S.A. Barker*, J.L. Linton*, and S.T. Christian. Neurosciences Program, Univ. of Ala. in Birmingham, Birmingham, AL 35294.

We have utilized 1-acyl-2-(N-4-nitrobenzo-2-oxa-1,3-diazole)-amino-dodecanoyl phosphatidylcholine (I), as a substrate for measuring phospholipase A_2 (PLA_2) (E.C.3.1.1.4) activity in Naja-naja venom. Hydrolytic activity was quantified by: 1) measuring the fluorescence of NBD-aminododecanoic acid (II) formed during incubation of PLA_2 and I, after separation via thin-layer chromatography, and 2) real-time measurement of fluorescence polarization. The decrease in fluorescence polarization was concomitant with increased formation of II, and was linear over a 1,000 sec interval after addition of the enzyme source, with detectable changes occurring within the first 120 sec. The optimum pH for enzymatic activity was approximately 9. Enzymatic activity was stimulated by Ca^{++}, and inhibited by EGTA. By using a real-time polarization assay, phospholipase A_2 activity can be measured rapidly, and precisely, at low concentrations of both enzyme (~ 0.01 µg) and substrate (3.5 µM). Preliminary experiments indicate that this technique might also be suitable for studying the kinetics of lipid-protein association in general.

64.19 CHRONIC EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) PRODUCED BY PROTEOLIPID APOPROTEIN: IMMUNOLOGIC STUDIES. F. CAMBI*, M.B. LEES and R.M. WILLIAMS*. E.K. Shriver Ctr., Waltham, MA. 02254.

Chronic EAE, induced by immunization with whole neural tissue, is a useful model for understanding the pathophysiology of demyelination and the immune mechanisms involved. Myelin basic protein (MBP) does not account for all the clinical and histological features of chronic EAE and it is recognized that other antigens are required to elicit demyelination. Proteolipid protein is present in CNS myelin in amounts as great as or greater than MBP. The proteolipid protein is an intrinsic membrane protein which has a portion of the molecule at the external surface of the myelin lamellae. It is therefore partially in contact with the extracellular environment where immune factors can play a role. In the present study we have investigated the encephalitogenic role of this protein in the production of chronic EAE. Twelve rabbits were each immunized with 1 mg of the water-soluble form of bovine proteolipid apoprotein (APL) plus complete Freund's adjuvant. In eleven animals either a progressive or relapsing chronic EAE developed one to six months post-immunization. The clinical course was characterized by posterior ataxia and flaccid paralysis progressing to spastic paralysis and various degrees of bladder dysfunction. Light and electron microscopic observations showed both acute and chronic encephalomyelitis accompanied by primary demyelination. The lesions, localized mainly in the spinal cord and optic nerve, were consistent with those seen in chronic EAE induced by whole CNS tissue.

Immunological studies were carried out to assess both humoral and cellular immunity. Anti-APL serum antibody production was determined by both an ELISA and an electroblot procedure. The two methods gave essentially the same results. Antibodies to APL were detected in 8 rabbits during the course of the disease but antibody titer did not correlate with either the clinical course or the histopathological pattern. To detect the presence of cell-mediated immunity, delayed-type hypersensitivity was assayed by skin-tests. A positive reaction to APL appeared in all rabbits prior to the onset of clinical signs. In 11 animals a response could be detected as early as 20 days after immunization. The additional rabbit showed a positive skin test at 2 1/2 months. All controls were negative. These findings suggest a pathogenetic role for cell-mediated immunity in the development of the demyelination. Immune attack on the proteolipid could alter lipid-protein interactions that maintain the integrity of the myelin lamellae.

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64.20 INDUCTION OF SULFOLIPID SYNTHESIS IN CULTURED C-6 GLIAL CELLS. Bhat, N.R.* and Volpe, J.J. Depts. of Ped., Neurol., Biol. Chem., Washington Univ. Sch. Med., St. Louis, MO 63110

C-6 glial cells in culture have been shown to exhibit biochemical expressions of oligodendroglial or astrocytic differentiation. Moreover, the specific nature of the expression of differentiation appears to relate in part to the age in culture (Parker et al., 1979). Thus, a cell density-dependent induction of the oligodendroglial markers, 2',3'-cyclic nucleotide phosphohydrolase (CNPase), has been observed in cells of early passages, whereas a density-dependent induction of the astrocytic marker, glutamine synthetase, has been observed in cells of later passages. A more readily defined manipulation for the induction of CNPase in C-6 glia has been shown by our previous study (Maltese and Volpe, 1979) to be the removal of serum from the culture medium. In the present study, we have utilized this manipulation to determine (1) whether sulfolipid synthesis, a particularly characteristic feature of oligodendroglial differentiation, is also stimulated, and (2) whether any such stimulation is related to the age of cells in culture.

When serum was removed from the medium of cells of early passage (e.g., 20 or less) a striking 6-8-fold increase in sulfolipid synthesis (incorporation of $H_2{}^{35}SO_4$) was observed after 48 hours. Serum removal had no effect on arylsulfatase A activity, thus suggesting that the enhanced labeling of sulfolipid reflected a true increase in synthesis rather than reduced degradation. Moreover, the increased incorporation of ^{35}S into sulfolipid was shown not to be accompanied by any change in uptake of the radioactive precursor or in the incorporation of label into sulfated mucopolysaccharides. The latter observation suggested that the mechanism of the enhanced incorporation of ^{35}S into sulfolipid involved a step subsequent to the formation of "active sulfate", i.e., adenosine 3'-phosphate, 5'-phosphosulfate (PAPS). All of these observations suggest that serum removal leads to enhanced synthesis of sulfolipid by an increase in activity of cerebroside: PAPS sulfotransferase. Our current studies are directed at confirmation of this notion. Finally, the stimulatory effect of serum removal on sulfolipid synthesis was shown to be clearly dependent on the age of cells in culture. Thus, in contrast to the effect in cells of early passage, only minimal incorporation of ^{35}S into sulfolipid could be detected in the presence or in the absence of serum in cells of late passages (e.g., 60 or more).

Thus, when coupled with our previous studies of CNPase, these data suggest that serum removal is a potent stimulus to oligodendroglial differentiation of cultured C-6 glial cells. Moreover, the effectiveness of this stimulus is highly dependent on the age of the cells in culture.

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65.1 INDUCTION OF ORNITHINE DECARBOXYLASE DURING RECOVERY FROM CEREBRAL TRAUMA. Gerald A. Dienel and Nancy F. Cruz* Dept. Neurology Cornell Univ. Medical College. New York, N.Y. 10021

Metabolic, mechanical, thermal, and chemical trauma induce ornithine decarboxylase (ODC) activity in rat brain. Cerebral ischemia, a severe metabolic injury, was produced by cauterization of the vertebral arteries and reversible clamping of the common carotid arteries for 30 min.; recirculation occurs after removal of the carotid cuffs. During the early phase (5h) of postischemic recirculation, when the rate of protein synthesis was reduced by about 50%, ODC activity was induced 6-8-fold; the rise in activity continued to 15-30 times normal at later stages (10-12h) of recovery (Brain Res (1975) 95, 61-73; Fed. Proc. (1982) 41, 646). The increase in ODC activity corresponded to the increase in V_{max} for ODC, and could be prevented by inhibition of protein synthesis with anisomycin, suggesting preferential synthesis of ODC during postischemia. Not all short half-lived proteins were preferentially synthesized. S-Adenosylmethionine decarboxylase (SAMDC) activity was reduced by 50-70% during the span from 1-36h postischemia, and proteins with longer half lives, acetylcholinesterase, lactate dehydrogenase, and total and 17000g supernatant protein were unchanged at 5 and 15h. Induction of ODC activity during recovery from 30 min transient cerebral ischemia also occurred in the liver (421±100(4)% at 5h, 185±68(4)% at 9h), but not in spleen (110±1(4)% at 5h, 60±1(4)% at 9h). Intracerebral injections of actinomycin D (Act D, 1 mg/ml in 220 mM mannitol) reduced the postischemic rise in ODC activity from 484±43(7)% to 339±103(4)% at 5h. However, the injection procedure caused sufficient thermal (drilling burr holes) and mechanical (needle track and injection pressure) trauma to induce ODC 5h later: injection of mannitol (220 mM, 10 µl/site x 4 sites), 571±88(4)%; drilling 4 burr holes without puncturing the dura, 282±39(4)%; hyperthermia (rectal temp. to 42°C, 15 min.), 200±14(4)%. Hyperthermia produced larger increases in ODC activity in liver (481±58(7)%) and spleen (813±161(7)%) than in the brain; it did not alter SAMDC activity in any of these three tissues. Both hyperthermia and ischemia are known to produce polysome disaggregation, thereby impairing protein synthesis. Recovery (8h) from inhibition of protein synthesis with anisomycin (100 mg/kg) was accompanied by increased ODC activity in the cerebral cortex (545±259(4)%). An ammonia load depresses protein synthesis in portacaval shunted rats; recovery from an ammonia injection (1.5 mmol NH_4 acetate/kg) showed increased ODC activity in cerebral cortex (259±35(6)%). Cellular response to noxious or stressful stimuli, such as metals, SH reagents, heat, anoxia, etc. includes the synthesis of a small number of proteins of unknown functions; ODC may be one of these proteins.

65.2 FAILURE OF NALOXONE TO LIMIT CLINICAL OR MORPHOLOGICAL BRAIN DAMAGE IN GERBILS WITH UNILATERAL CAROTID ARTERY OCCLUSION. D.E. Levy, C.L. Pike*, and D.G. Rawlinson*. Department of Neurology, Cornell Univ. Med. College. New York, NY 10021.

Naloxone has been reported to reverse ischemic dysfunction in man (Lancet ii, 272-275, 1981) and in gerbils subjected to unilateral carotid artery occlusion (Science 215, 69-71, 1982). To clarify in perfusion-fixed brains, the extent of morphological benefit, we repeated the study in gerbils.

Under halothane anesthesia, gerbils underwent placement of a reversible plastic clasp around the right common carotid artery and were then permitted to recover. One day later, these fasted animals were briefly restrained, and the carotid clasp was tightened. Clinical observations were made every minute, and by the end of 10 min, gerbils were classified as clinically-affected or unaffected in accordance with previously-published criteria (J. Neurol. Neurosurg. Psychiatr. 38, 1197-1205, 1975). At that time, clinically-affected gerbils received either naloxone (1 mg/kg I.P.) or an equivalent volume of saline; the individual observing clinical behavior was unaware of the agent administered. Clinical observations were continued for a total ischemic interval of 1 hr, after which the carotid clasp was removed. Animals were permitted to survive for 24 hrs and were then killed by perfusion-fixation with 40% formaldehyde-glacial acetic acid-absolute methanol (1:1:8). Brains were removed after at least 4 hrs, the forebrains were sectioned in anterior and posterior coronal planes, and stained sections were graded independently (0 to 4+ scale) for the severity of ischemic damage in the cerebral cortex, hippocampus, thalamus, and striatum.

Forty gerbils were subjected to unilateral occlusion, and 14 (35%) were clinically-affected within the first 10 min. Nine of these affected animals received naloxone, and 5, saline. There was no detectable clinical affect from the agent in any of the 14 animals, either during or following the ischemic interval. One of the naloxone-treated animals died at approximately 18 hrs. In the 13 survivors, the severity of ischemic brain damage was similar in all brain regions in naloxone- and saline-treated animals (Mann-Whitney U Test).

These results contrast sharply with those reported by Hosobuchi (Science). One possible explanation for the difference is that barbiturate anesthesia (which can in part be counteracted by naloxone) had been used in the gerbils studied by Hosobuchi, whereas no anesthetics were given on the day of the experiment in our animals. Whatever the explanation, the circumstances under which naloxone produces its beneficial effect need clarification.

65.3 NALOXONE'S EFFECT ON REVERSING HYPERIRRITABILITY IN THE EARLY STAGES OF STROKE IN THE SPONTANEOUSLY HYPERTENSIVE STROKE-PRONE RAT (SHR-SP). V.K. Dullien*, W.J. Giardina and J. Wiese, Abbott Laboratories, North Chicago, IL 60064.

A colony of SHR-SP was maintained after weaning on high salt [HS] (8% NACl) chow and ad lib water. Under standard laboratory diet conditions these rats did not develop spontaneous stroke, but with the HS diet a high incidence of stroke occurred in this colony. During the 4th month of life, 76% of the males on HS (N=55) developed stroke compared to 2% on a standard diet (N=48). Stroke was confirmed at autopsy by gross and histological analysis. Progression of behavorial stroke symptoms agreed with the reports of Yamori et al. (1). The early stage of stroke was characterized by marked hyperirritability to arousal stimulus and hyperactivity with jumping and escaping behavior. Later stages of stroke are characterized by hyporesponsiveness to arousal stimulus and lethargy.

Recent papers in the literature (2) report that naloxone can temporarily reverse stroke signs. We tested the effects of naloxone 1 mg/kg, i.p. in the early stage of stroke in 3-4 month old male SHR-SP. We observed temporary reversal at 1 hour post-injection of naloxone of hyperirritability (biting) to arousal stimulus (a gloved hand) and hyperactivity (jumping, escaping). Eighty-two percent of the naloxone animals (N=11) showed reversal to normal behavior compared to 11% of saline controls (N=9). At 15 minutes post-injection, reversal of behavorial stroke symptoms had not yet occurred and by 24 hours post-injection the naloxone rats longer exhibited reversal of stroke symptoms. During the pre-stroke stage (2.5 months of age) there was no change in the normal response to arousal stimulus or activity with either saline injection (N=11) or naloxone injection (N=11). We conclude that naloxone has a significant effect on temporarily reversing the hyperirritability and hyperactivity associated with the early stage of stroke in SHR-SP. Naloxone given to SHR-SP prior to the onset of stroke symptoms did not produce a sedative effect which could account for the reduction in hyperirritability of early stroke.

References:
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65.4 ELEVATED GROWTH HORMONE PLASMA LEVELS IN HUNTINGTON'S DISEASE. BASAL AND STIMULATED CONDITIONS. C.A. Tamminga, R. Durso*, S. Ruggeri*, A. Denaro*, S. Kuo* and T.N. Chase. Pharmacology Section, ETB, IRP, NINCDS, NIH, Bethesda, MD 20205.

Huntington's disease is a dominant inherited disorder characterized predominantly by motor dysfunction, cognitive deterioration, and premature death. Basic and clinical research have focused on those areas of brain intimately and obviously involved in symptom expression, namely the basal ganglia and cortex. Although not intensively studied previously, there is evidence that the hypothalamus is pathologically affected in HD as well. The hypothalamus modulates hormonal function in the pituitary gland; thus, pathology within the hypothalamus can be reflected in abnormalities of anterior pituitary hormone levels in plasma. Our goals have been to derive evidence of functional abnormalities in the HD hypothalamus, which could mark the onset or progress of the syndrome or reveal aspects of its pathophysiology.

We have tested plasma from 9 women with HD and matched female controls for growth hormone (GH), prolactin (Prl), and luteinizing hormone (LH). Samples were drawn every 30 minutes over 24 hours from an indwelling, heparinized catheter and sleep was monitored by EEG. Blood samples were drawn, placed immediately on ice, separated within one hour and the plasma frozen at -70°C until time of assay. The samples were analyzed by radioimmunoassay using the materials and methods supplied by the National Pituitary Agency. Analysis of variance confirmed a significant elevation of plasma GH in the HD subjects over the 24 hour time period. Additionally, mean 24 hour levels of GH were significantly higher in the HD subjects than in the control individuals (4.44 ng/ml ±. 39 and 2.66 ng/ml ± .34, respectively). Stress and exercise were examined to account for the GH elevation but were ruled out. Prolactin levels over 24 hours and mean 24 hr values were not significantly different between groups. Further pharmacologic studies to be reported show that HD subjects have an exaggerated GH response to the administration of both apomorphine (0.75 mg subcutaneously) and muscimol (5mg, orally), but a normal response to arecoline. These basal and stimulated neuroendocrine studies suggest a hypothalamic disinhibition of GH release more generalized than that produced by a single neurotransmitter system abnormality. Loss of the hypothalamic GH inhibiting factor, somatostatin could explain these results.

65.5 HUNTINGTON'S DISEASE AND L-PYROGLUTAMIC ACID: A BEHAVIORAL, ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL EVALUATION OF THE POSSIBLE ROLE OF THIS IMINO ACID IN HUNTINGTON'S DISEASE, G.K. Rieke and J.F. Hunter*. Dept. of Anatomy, Coll. of Med. & Dept. of Vet. Physio. & Pharmacol., Texas A&M Univ., College Station, TX 77843.

Huntington's disease (HD) is a progressive neurodegenerative disorder that afflicts both sexes and is transmitted as an autosomal dominant with complete penetrance. The causes of nerve cell destruction in HD are not understood. The kainic acid (KA) rodent model of HD, introduced by Coyle and Schwarcz (1976), has led to the hypothesis that a KA-like substance is present in HD patients. KA is a substituted pyrrolidine ring and an imino acid that is present only in plant tissue. An imino acid detected in the plasma of HD patients (Perry et al., 1981) mimicked KA during automated amino acid analysis. L-pyroglutamic acid (L-PGA) structurally resembles KA and it is an imino acid, and an intermediate in the γ-glutamyl cycle. Intrastriatal injections of L-PGA (10 mM, 1 mM or 0.1 mM, pH 7.3-7.4, 2 μL total volume) in mouse produce a rotatory (circling) response and postural asymmetry. The experimental animals circled toward the side of the injection at rates that were significantly higher than the control group receiving equal volumes of the saline vehicle (0.9% NaCl) alone. Postural asymmetries involved the head and trunk. The head was rotated downward and toward the side of the injection and the trunk showed a lateral bowing. A tremor (>10 Hz) was observed and animals showed sporadic, coarse snapping movements of the head and trunk, but no seizure activity. The discharge rates of neurons sensitive to L-PGA increased when these cells were challenged by extracellular injections of 0.2 μL aliquots of 1 mM L-PGA (pH 7.3) and the increased rate (≈5 times the baseline rate) was followed by a sudden cessation of firing. L-PGA destroys the soma and associated dendrites of neurons in the injected mouse caudato-putamen (CPU). The neuropil contained vacuoles and swollen profiles (dendritic or glial) by 72 hr after the injection of L-PGA. The mitochondria and endoplasmic reticulum of sensitive neurons were dilated and many of the mitochondrial cristae were obliterated. Dilated dendritic profiles and degenerating somal spines were associated with intact presynaptic terminals complete with synaptic vessicles. Myelinated profiles within the injected CPU remained intact even 60 days after the injection. Reactive microglial-like cells were present in the neuropil by 72 hr and by 60 days phagocytic cells were prominent. Our data show that L-PGA is an excitatory and neurotoxic imino acid. Since L-PGA is a significant metabolite of glutathione (Meister, 1978), a metabolic error (altered enzyme) in the γ-glutamyl cycle might lead to subtle yet accumulative increases in intracellular levels of L-PGA and ultimately cell death.
Supported by Center for Comp. Med. (18820), Texas A&M Univ.

65.6 PREVENTION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS WITH A NONTOXIC DERIVATIVE OF COBRATOXIN. I. N. Montgomery[1]*, R. A. Hudson[2]* and H. C. Rauch[1]. Depts. Immunol./Microbiol.[1] and Biochemistry[2], Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Treatment with a carboxamidomethylated (CAM) non-toxic derivative of the principal curarimimetic neurotoxin derived from the venom of the Thailand cobra, Naja naja siamensis, protects guinea pigs (gps) from experimental allergic encephalomyelitis (EAE). In contrast to untreated control animals which develop EAE within 14 to 21 days following challenge with myelin basic protein (MBP), those treated with the CAM-neurotoxin showed no signs of disease. Dermal delayed type hypersensitivity (DTH) to MBP following challenge was significantly reduced in neurotoxin-treated gps. In control gps challenged with MBP but not treated with CAM-neurotoxin, dermal DTH response to both CAM-neurotoxin and MBP indicated immunological cross reactivity at the cellular level. Humoral cross reactivity was indicated following the infusion of MBP by the development of passive cutaneous anaphylaxis (PCA) at intradermal deposits of sera obtained from neurotoxin treated gps. Competitive binding with ^{125}I-alpha-bungarotoxin was observed between the synthetic encephalitogenic sequence of MBP (residue 114-122) and the acetylcholine receptor (AChR). We suggest that the molecular basis for the relationship between these proteins is centered in their respective tryptophan-containing regions, and that interaction of the CAM-neurotoxin at immune cell membrane receptor(s) may be the basis for its protective action.

This research was supported in part by NIH grants NS-14491 and NS-12754.

65.7 DISTRIBUTION OF MYELIN-ASSOCIATED GLYCOPROTEIN (MAG), MYELIN BASIC PROTEIN (MBP) AND GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IN CHRONIC RELAPSING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE). H. Shii,* H. deF. Webster and H. Lassmann*. NINCDS, NIH, Bethesda, Maryland, and Neurological Institute, University of Vienna, Vienna, Austria.

To study the mechanism of myelin breakdown and regeneration in chronic relapsing EAE, we used antisera to MAG, an oligodendroglial constituent, MBP, a major component of CNS myelin, and astrocytic GFAP to immunostain paraffin and epon sections of lesions according to the peroxidase-antiperoxidase method. In lesions with histological evidence of active myelin breakdown, perivascular mononuclear infiltrates were present, the areas of decreased or absent MAG and MBP staining corresponded closely, and GFAP staining revealed little astrocytic hypertrophy. Astrocytes and macrophages contained many more MBP than MAG stained breakdown products. In more chronic lesions, MAG and MBP antisera stained thin regenerated myelin sheaths and some nearby oligodendroglia. Astrocytic processes were intensely stained by GFAP antiserum; they were larger and more numerous. There were relatively few mononuclear cells and macrophages; the latter contained some MBP stained myelin remnants. These results suggest that myelin is the primary target in the demyelination associated with chronic relapsing EAE. Oligodendroglia are relatively spared and quickly remyelinate many of the demyelinated axons. Our observations confirm others (Eng, 1980) and show that GFAP immunostaining is a sensitive method for demonstrating astrocytic hypertrophy.
(This research was supported in part by a grant from the Kroc Foundation).

65.8 PERSISTENCE OF ANTIBODIES TO GM1 GANGLIOSIDE IN BLOOD AFTER INTRACEREBRAL INJECTION. MECHANISM OF SEIZURE INDUCTION. S.E. Karpiak, S.P. Mahadik, V. Ciccarone* & M.M. Rapport. Div. of Neuroscience, NY State Psychiatric Inst. and the Depts. of Psychiatry and Biochemistry, College of Physicians & Surgeons, Columbia Univ., New York, N.Y. 10032.

Intracerebral injection of antibodies to GM1 ganglioside causes specific alterations in CNS functions including induction of recurrent epileptiform spiking (1,2), inhibition of the consolidation phase of learning(3), inhibition of morphine analgesia(4), etc (5). We are studying the localization and persistence of such antibodies in vivo. Rabbit antibodies to GM1 ganglioside were purified by affinity chromatography, separated into IgG and IgM classes and labelled with [125]I. Rats were injected intracortically with these labelled anti-GM1 ganglioside antibodies and labelled rabbit "normal" IgG as a control (12ug protein, 1.9 to 2.5 million cpm). The levels of radioactivity measured in venous blood at 1,6,24 and 48 hrs showed the following. Antibodies to GM1 entered the blood from the brain much more slowly than "normal" IgG; IgM antibodies were retained much longer than IgG antibodies. The level of radioactivity in the blood from intracerebral injection of both "normal" IgG and antibody IgG peaked at 24 hrs, whereas that from IgM antibody remained high at 48 hrs. Although radioactivity associated with antibody IgG cleared from blood more rapidly than "normal" IgG in the 24 to 48 hr period, the level at 48 hrs was still 15 to 25% of that at 24 hrs. These results support the concept that the delayed seizure induction seen in the immunoneurological model of epilepsy may result from "reinvasion" of the brain at the site of injection by antibodies circulating in the blood, and are consistent with autoradiographic studies(6).

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2. Karpiak et al., Epilepsia 22:189 (1981).
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Supported in part by a grant from USPHS (NS-13762).

65.9 DISCRETE LESIONS IN THE BRAIN PRODUCED BY LINES OF T LYMPHOCYTES REACTIVE AGAINST EXOGENOUS ANTIGENS. S. Jacobson, J. Liran, S. Eisenstein, J. Holoshitz, Y. Naparstek, A. Ben-Nun and I.R. Cohen. Department of Cell Biology and the Center for Neurosciences and Behavioral Research, The Weizmann Institute of Science, Rehovot, Israel and Department of Anatomy, Tufts University, School of Medicine, Boston, MA.

Generalized inflammatory lesions in the brain and other organs have been produced in rats by inoculating the rats intravenously with cells of T lymphocyte lines and then injecting the target organ with the specific antigen to which the line cells are uniquely reactive (Y. Naparstek, J. Holoshitz, A. Ben-Nun and I.R. Cohen, in preparation). We have now used this technique to produce discrete lesions in the brain. Lewis rats were inoculated with 10^7 activated cells of a T lymphocyte line reactive to ovalbumin (OA) and 2 weeks to 2 months later the rats were injected stereotoxically with 5 and 10 µl of 10% OA in PBS several cortical and sub-cortical sites. The rats were killed at various times thereafter by infusion of 10% neutral buffered formalin and their brain were studied histologically. Infiltration of mononuclear cells was seen at the sites of injection. Neuronal and glial elements were observed to undergo degeneration and phagocytosis several days later and by two weeks there remained only a discrete cavity lined by astrocytic cells of about 1 mm.

Thus, it is possible to direct T lymphocytes to a discrete area of the brain and produce a limited lesion by injecting the specific antigen into the site. This observation has implications for immunological disease of the nervous system. It also provides a new tool for producing anatomically defined brain lesion and then for studying the resultant functional (behavioral and physiological) disturbances.

65.10 CSF PRODUCTION RATE AND INTRACRANIAL COMPLIANCE IN A NEW STRAIN OF HYDROCEPHALIC RATS. M.E. Miner, L. Young*, D. Drennon*, D. Kohn*, S. Graham* and L. Hackenberry*. Dept. of Neurosurgery, Univ. of Texas Med. Sch., Houston, TX 77030.

We investigated a new strain of rats which have a polygenic inheritance of communicating hydrocephalus. The hydrocephalic animals can be identified from their unaffected litter mates by visual inspection of the newborn animals. The hydrocephalic animals live for 4 to 5 weeks, then their weight falls off rapidly, they become progressively less responsive and die. We propose there is an increase in resting spinal fluid pressure and a decrease in compliance in these hydrocephalic animals.

Nine Sprague-Dawley rats (S-D), seven unaffected rats from the hydrocephalic colony (N), and ten hydrocephalic rats (H) were studied. A needle was inserted into the spinal subarachnoid space of each anesthesized animal and connected to a pressure transducer and a microsyringe pump. Every two seconds the CSF pressure data were digitized and stored by a microcomputer. Saline was infused at 0.0025, 0.0068, 0.013, and 0.026 cc/min, each infusion lasting eight minutes. Spinal fluid opening pressure (PO), spinal fluid production rate, and intracranial compliance were measured or calculated in each animal.

PO (in mmHg) was the baseline reading prior to infusion of saline. The average PO of S-D, N, and H was 2.03 mmHg±0.46, 2.43 mmHg±.32, and 3.74 mmHg±0.36, respectively. A difference ($p<0.05$) was observed between S-D and H and between N and H, but not between S-D and N.

The calculated CSF production rates in the S-D (3.34cc/min±1.26), N(2.88cc/min±0.62) and H(3.88cc/min±1.07) were not significantly different.

Compliance (C) was calculated and the significant differences were between S-D and N ($p<0.1$) at an infusion rate of 0.026 cc/min where the mean C_{S-D} was 1.02E-04±2.9E-04 and that for C_N was 1.35E-03±3.94E-04; also between S-D and H ($p<0.1$) at a rate of .026cc/min where the means were 1.02E-04±2.9E-04 and 7.1E-04±2.57E-04; and also at a flow of 0.0068 cc/min between N and H ($p<0.05$) where the means were 8.4E-04±6.5E-05 and 5.29E-04±1.17E-04.

The hydrocephalic rats had higher opening pressures than litter mates or normal rats. Intracranial compliance tended to be higher in the hydrocephalic rats at high flow rates than their litter mates, but their compliance also tended to be higher than normal rats. The CSF production rate was the same in each group. This suggests that the excessive CSF is due to decreased CSF absorption rather than over-production.

65.11 THE EFFECTS OF TOXOCARA CANIS UPON ODOR-DISCRIMINATION IN MICE. D.E. Yuhl, P.J. Donovick, R.G. Burright*, and N.E. Spear. Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, NY 13901 and R.H. Cypess*, Dept. of Preventive Med., Cornell Univ., Ithaca, NY 14850.

Toxocara canis, a parasitic nematode, is estimated to infect as many as two-thirds of all dogs, its natural host. The eggs of the parasite, passed in dog feces, are practically invulnerable to aversive environmental conditions, and can be ingested by various organisms, including mice and humans. In such aberrant hosts, T. canis eggs develop only to a larval stage, and migrate to a variety of somatic tissues including liver, lungs, heart, eyes, and CNS. Indeed, T. canis is considered the principal agent of visceral larva migrans, a heterogeneous group of clinical disorders resulting from the migration of larval parasites through visceral organs in abnormal hosts such as humans. The widespread prevalence of T. canis eggs, coupled with the difficulty of diagnosing and treating toxocariasis in humans, demonstrates the need for information concerning the consequences of this potential health hazard.

Since previous studies in our lab have reported that dramatic behavioral changes are associated with toxocariasis in mice, the present study was conducted to examine the effects of T. canis infection in mice on their learning and retention of a classically conditioned odor discrimination. The animals were gastrointestinally intubated with approximately 900 viable T. canis eggs, and 4 weeks post-intubation they received pairings of Odor A with footshock and Odor B with no footshock. Relative preference for these odors was measured immediately after the training, and at retention intervals of 3, 7, and 21 days. The mice were sacrificed at the end of testing, and post-mortem examinations were conducted to determine the number and location of the larvae in the brain. Transmission electron microscopy is presently being conducted on various body organs.

As measured by odor preference, T. canis infection did not significantly impair initial conditioning of the odor aversion, but thereafter, the conditioned discrimination deteriorated more rapidly for the parasite burdened mice than for the controls. This difference could not be attributed to gross olfactory deficits from T. canis infection, indicating that the effect of this parasite was either to enhance forgetting or to decrease resistance to extinction. Histopathology revealed no differences in distribution of the larvae in the cerebral hemispheres and cerebellum, but, as noted in earlier studies, the larval parasites exhibited a predilection for myelinated fiber tracts. The potential for similar change in the performance of learned tasks by humans must be considered.

65.12 EFFECT OF Pco_2 ON REGIONAL CEREBRAL BLOOD FLOW DURING BARBITURATE AND HALOTHANE ANESTHESIA. H.L. Edmonds, Jr., M.K. Halbert*, U.M. Kayerker* and K.K. Sawhney*. Dept. Anesthes., Univ. Louisville, Louisville, KY 40292

The present study, employing radioactive microspheres to determine cerebral blood flow (CBF), compared regional cerebrovascular responses to changes in Pco_2 in dogs anesthetized with pentobarbital (PB) or halothane (HAL). After anesthetic induction and surgical preparation changes in respiratory rate and tidal volume served to vary Pco_2 over a range of 22 to 55 torr. At the end of the experiment, animals were terminated with an overdose of KCl. Cortex (CTX), diencephalon (DIE), brain stem (BS) and cerebellum (CB) were dissected, weighed, desiccated, reweighed and counted by gamma spectroscopy to determine CBF. Flow was calculated both in absolute terms of ml/min/g wet tissue (CBFa) (see table below) and as a percent of cardiac output (CBF%).

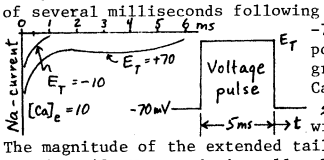
Linear regression of total CBFa on Pco_2 yielded correlation coefficients of 0.46 for PB and 0.89 for HAL. Similar results were obtained for regressions using each brain region and CBF% instead of CBFa. The strong dependency of CBF on Pco_2 in the HAL group necessitated separate calculation of mean values for dogs with a low Pco_2 (< 30 torr) and high Pco_2 (> 40 torr.

	PB	HAL hi CO_2	HAL lo CO_2
n	6	5	4
CTX	.42±.09	1.79± .21	.54±.07
DIE	.44±.05	3.54± 1.14	.59±.18
BS	.44±.05	2.27± .23	.56±.13
CB	.60±.12	2.49± .22	.63±.13

These results are consistent with previous findings that, compared to barbiturate anesthesia, HAL enhances cerebrovascular reactivity to changes in Pco_2. Sensitivity of cerebral blood vessels to CO_2 may not be uniform, but instead vary regionally. Diencephalic vessels seem most responsive to CO_2 perturbation. However, the apparent differences in vascular reactivity may be a function of the method of CBF measurement. Lung blood flow in HAL treated dogs was abnormally high (especially during hypercapnia) indicating non-entrappment of the 15 µm spheres in some other tissues. Supported, in part, by the Distilled Spirits Council of the U.S.

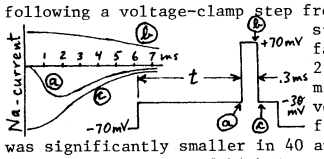
66.1 SLOW AND MICROSECOND KINETICS OBSERVED IN THE SODIUM CHANNEL ACTIVATION GATING. J.F.Fohlmeister and W.J.Adelman. Lab of Biophysics, NINCDS,NIH,Marine Biol. Lab., Woods Hole MA 02543 and Department of Physiol.,Univ of Minnesota, Minneapolis MN 55455.

With the sodium channel of the perfused squid giant axon isolatedby the use of K-free solutions containing only Na^+ and Cs^+ cations (plus sucrose) internally and Na^+,Cs^+ and Ca^{++} cations externally, the sodium current was observed to remain on for a period of several milliseconds following voltage-clamp repolarisation to



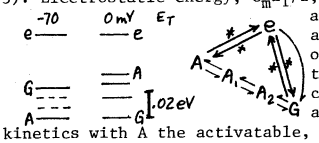
-70 mV (fig 1) provided the test potential,E_T, was significantly greater than 0 mV and low external Ca (2 to 10 mM) was used. With $E_T \lesssim 0$ mV the current returned to ~0 with time constant ~.41 ms (5° C). The magnitude of the extended tail currents was significantly reduced in 40 mM Ca and virtually absent in 100 mM Ca, returning to ~ zero on the scale of .41 ms.

In a related set of experiments using the same ionic solutions the Na-conductance was observed to abruptly change within 25 ms following a voltage-clamp step from +70 to -30 mV. The conductance



step amounted to a reduction by a factor of ~2 for t=4.5 to 7 ms in 2 to 10 mM Ca^{++} based on currents measured at points ⓑ and ⓒ of the voltage-clamp pulse profile (fig 2) for a series of times t. The step was significantly smaller in 40 and 100 mM Ca^{++} although the channel blocking action of high Ca on the inwardly directed Na-current makes a conductance determination at -30 mV somewhat unreliable at these Ca- concentrations. Due to the presence of transients the current at point ⓒ was taken 25 ms following clamp-step initiation.

The effects may be explained by including microsecond transitions (*) to a closed excited state,e, in the gationg kinetics (fig 3). Electrostatic energy, $C_m E_T^2/2$, released during clamp-steps,



amounts to ~150 electronVolts/μm^2 at E_T=70 mV; this is 1 to 2 orders of magnitude above thermal when the energy is divided among the channels. Transitions $A \rightleftharpoons A_1 \rightleftharpoons A_2 \rightleftharpoons G$ are Hodgkin-Huxley activation kinetics with A the activatable, and G the open state. In the model kinetics, initial depolarisations (-70 to $\gtrsim$0 mV) lead to $A \xrightarrow{*} e$ and repolarisations (+70 to $\lesssim$0 mV) lead to $G \xrightarrow{*} e$ which results in a sudden depletion of G (with reduced conductance). The postulated transition $e \longrightarrow G$ is slow (~3.3ms), resulting in the extended tails. On the basis of the data, state e is relatively inaccessible in high Ca^{++}.

66.2 MODIFICATION OF SINGLE SODIUM CHANNELS BY THE INSECTICIDE TETRAMETHRIN. Daisuke Yamamoto*, Fred N. Quandt and Toshio Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., 303 E. Chicago Ave., Chicago, IL 60611.

The gigaohm sealing, patch clamp technique has recently been applied to the voltage dependent sodium channel. We applied this technique to determine how properties of the conducting state of the sodium channel are modified by the insecticide tetramethrin, a synthetic analog of the naturally occurring insecticide pyrethrins. Previous studies with crayfish and squid giant axons revealed that tetramethrin caused a prolongation of the sodium current (A.E. Lund, T. Narahashi, Neurotoxicology 2, 213-229, 1981; J. Pharmacol. Exp. Ther. 219, 464-473, 1981).

Sodium channel activity was recorded from membranes of neuroblastoma cells (N1E-115) at low temperatures (9-12.5°C). The glass pipettes used for patch clamping had an internal tip diameter of 0.3-0.8 μm with resistance of 2-10 megohms. The seal resistance was 5-10 gigaohms. After forming a seal, a cell-free membrane was isolated to obtain either an "inside-out" or an "outside-out" patch. (+)-trans tetramethrin was applied to the internal membrane surface.

Depolarization of the patch membrane to -50 mV or less negative potentials from a holding potential of -90 mV generated pulse-like inward currents due to the opening of sodium channels. The mean amplitude at -50 mV was approximately 1.5 pA, and the conductance calculated from the change in current size at different protentials was about 10 pS. A Poisson plot of the open times of the conducting states indicated a single exponential distribution. In a typical experiment, the mean rate constant for channel closure was 0.588 ms^{-1}. In the presence of tetramethrin (30-60 μM), the single channel conductance was not appreciably changed, whereas the distribution of open times in the Poisson plot was better fitted by a sum of two exponential functions. A portion of the population has a slower closing rate (0.059 ms^{-1}) than normal. The remaining exponential component had a rate constant of 0.526 ms^{-1}, which is close to that for the normal channels. Both components disappeared by external application of 3 μM tetrodotoxin, indicating that both populations of open states are associated with sodium channels. The prolongation of the channel open time for the modified population could be due either to elimination of the inactivation process or to a decrease in the reverse rate constant for opening of the channel. The sodium channel modified by tetramethrin appears to inactivate slowly because the open time decreases with large depolarizations. The lack of continued channel openings during maintained depolarizations also supports this notion. Supported by NIH grant NS 14143.

66.3 EFFECTS OF MYRMICACIN AND THEIR ANALOGOUS COMPOUNDS ON THE SODIUM CHANNEL IN THE SQUID GIANT AXON. T.TAKENAKA , H. HORIE and H. HORI* Dept. of Physiology, Sch. of Med., Yokohama City Univ., Minamiku, Yokohama, Japan 232

Myrmicacin and their analogous compounds inhibit the early inward-current in excitable membranes. The effects of these compounds on the inward-current of the nerve membrane was studied by using a voltage clamp in squid giant axon, *Doryteuthis bleekeri*. When myrmicacin and their analogous compounds (fatty acids 9 and 10 carbon atoms) were applied externally at the concentration of 300 ppm in artificial sea water, they depolarized the nerve membrane 2-5 mV and eventually blocked the action potencial. These effects were completely reversible by washing with artificial sea water. The membrane resistance was not changed by these chemicals. The maximum value of the early inward-current was decreased and reached the steady state value 15-20 min after application of them. These chemicals do not affect the late outward-current but only the early inward-current. These effects are reversed after washing with normal artificial sea water. Fatty acids less than 8 carbon atoms have no effect on either the early inward-current or late outward-current. Fatty acids with more than eleven carbon atoms are almost insoluble in artificial sea water. Next we compared with the effect of fatty acids of the same chain length. Sebacic acid, which has carboxyl groups at both ends of the chain, has no effect on the early inward-current or the resting potential. 10-hydroxy decanoic acid also has no effect on the early inward-current or the resting potential. 2-decenoic acid, which has a double bond between carbon atoms 2 and 3, was most effective fatty acid in our experiments. The same tendency toward inhibition of the early inward-current was observed when applied these compounds in the intracellularly perfused squid giant axon. These data show that the suppression of the Na-current depends of the number of carbon atoms in the compounds. However, it is not correct to conclude that the inhibitory activity depends only on number of carbon atoms, because sebacic acid and 10-hydroxy decanoic acid do not inhibit the Na-channel. Apparently the inhibitory activity of the compounds is also related to the chemical structure of the compounds. Recently we found that 2-decenoic acid increased the membrane fluidity. From these results it is possible to consider that myrmicacin and their analogous compounds get into the lipid layer of the nerve membrane and increased the membrane fluidity, which caused the perturbation of the membrane lipids and the Na-channel lipoprotein. These changes of the membrane might be the reason to suppress the Na-current of the nerve membrane by application of myrmicacin and their analogous compounds.

66.4 INTERACTIONS OF THE PYRETHROID FENVALERATE WITH THE NERVE MEMBRANE SODIUM CHANNEL. Vincent L. Salgado* and Toshio Narahashi (SPON: D.Harter). Dept. of Pharmacol., Northwestern Univ. Med. Sch., 303 E. Chicago Ave., Chicago, IL 60611.

The pyrethroid insecticides are very potent neuropoisons. The natural pyrethrins and most synthetic pyrethroids have the classical type I action characterized by excitation and tremors in animals and repetitive firing in nerves. The repetitive discharge is due to an increase in depolarizing after-potential which is in turn caused by a prolonged sodium current that follows the peak transient sodium current. The interaction of type I pyrethroids with the sodium channel has recently been analyzed in detail (A.E. Lund and T. Narahashi, Neurotoxicology 2, 213-229, 1981; J. Pharmacol. Exp. Ther. 219, 464-473, 1981). The synthetic pyrethroids containing a cyano group at the α position (type II) such as fenvalerate cause paralysis of the animals which is associated with nerve membrane depolarization and conduction block.

The crayfish giant axon was internally perfused and voltage clamped in a double sucrose-gap chamber. In an axon internally perfused with 1 μM fenvalerate ((S)-cyano-3-phenoxybenzyl (S)-2-(p-chlorophenyl)-3-methylbutyrate), a step depolarization from a holding membrane potential more negative than -100 mV produced a peak transient sodium current which partially inactivated. With prolonged depolarization there was a very slow, secondary rise in current with a time course of 1-5 sec without noticeable inactivation. The tail current associated with step repolarization of the membrane decayed extremely slowly with a time constant of 2.6 min at -100 mV and 0.27 min at -140 mV. Like the peak transient current, the slow and tail current were both blocked by 1 μM tetrodotoxin indicating that they flow through the sodium channel. These and other observations are compatible with a kinetic model originally developed for type I pyrethroids. In the presence of fenvalerate, the sodium channel opens normally and is modified by the pyrethroid. Fenvalerate also binds to the sodium channel at its closed state causing a modified closed channel. The latter opens slowly upon depolarization to generate a secondary slow current. The voltage dependence of activation of closed modified channels was shifted 20-30 mV in the direction of hyperpolarization. This, together with the absence of inactivation, causes a depolarization. It appears that type I and type II pyrethroids act on the nerve membrane sodium channel in a qualitatively similar manner. However, the opening and closing kinetics of the modified channel are much slower for type II than for type I pyrethroids.

Supported by NIH grant NS14143.

66.5 EFFECT OF VERATRIDINE ON MEMBRANE POTENTIAL AND SODIUM INFLUX IN FROG SKELETAL MUSCLE. L.C. McKinney. Dept. of Physiol. Biophys., Washington Univ. Med. Sch., St. Louis, Mo. 63110

The effect of the depolarizing agent veratridine (VER) on the steady state Na permeability of frog skeletal muscle was studied. Its effect was quantified in two ways: by measuring (a) resting membrane potential, and (b) influx of radiolabelled sodium. Sartorius muscles from Rana pipiens were used.

Membrane potential (Vm) was measured in Ringer solutions containing 2.5, 10, 30, 75, and 190 mM K ($[K][Cl] = 300\ mM^2$) and 10 mM ouabain in the absence and presence of 100 uM VER (Sigma and purified stocks). $[Na]_o$ = 10 mM. Vm vs. log $[K]_o$ curves were fit using the Goldman-Hodgkin-Katz equation with a single free parameter, $\alpha = P_{Na}/P_K$. VER causes α to increase 12.6 ± 1.2 fold (n = 9). The effect of VER on Vm is reversible ($T_{1/2}$ = 30 min), prevented by tetrodotoxin (TTX), sensitive to $[Na]_o$ and insensitive to curare.

Influx of ^{22}Na into whole sartorius muscles was determined by the method of Kennedy and De Weer (J.Gen. Physiol. 68:405, 1976). Muscle pairs were pre-exposed to 30 mM K Ringer plus 10 mM ouabain, and one muscle of the pair was then exposed to VER; both were then exposed to ^{22}Na. $[Na]_o$ = 10 mM unless otherwise specified. Vm = -37 mV. Only initial rates of Na uptake were measured. The time constant for the rate of action of 100 uM VER is 59 min. Raising external pH one unit to 8.3, which increases the concentration of the uncharged form of VER, causes the rate of action of VER and the level of Na influx in the presence of 100 uM VER to increase 1.2 and 1.5 fold respectively. VER-induced Na influx is completely inhibited by TTX, (K_d = 8 ± 2 nM). The apparent K_d for VER is voltage dependent: in 30 mM K/1 mM Na Ringer its value is 53 ± 14 uM; in 2.5 mM K/1 mM Na Ringer (Vm = -90 mV), it increases to 837 ± 284 uM. The magnitude of VER-induced Na influx increases at hyperpolarized potentials, and obeys the Goldman constant field flux equation. A saturating concentration of VER produces a P_{Na} of 1.5×10^{-7} cm/sec which is much less than the maximum possible P_{Na} (3.6×10^{-4} cm/sec) that can be calculated from measurements of peak Na conductance. This indicates that not all Na channels are opened by saturating concentrations of VER. The data are explained by a model of VER action whereby VER binds to both the open and inactivated forms of the sodium channel (Krueger and Blaustein, J. Gen. Physiol. 76:287, 1980).

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66.6 PROCAINE ACTIONS ON LEECH RETZIUS CELLS.
Jay Yang* and Anna L. Kleinhaus. Dept. of Neur., Yale U. Sch. of Medicine, 333 Cedar Street, New Haven, Ct. 06510.

We have previously shown that in leech segmental ganglia there are side by side cells sensitive to tetrodotoxin (TTX) and others resistant to the drug even at high concentration. The action potentials of both cell types are strongly dependent on Na (Neuroscience Abstr, 7:902, 1981).

We report here that procaine, a drug which is known to block Na conductance in a variety of excitable membranes, affects the action potentials of the TTX resistant R cell and of the TTX sensitive mechanosensory N cell, similarly.

Procaine (0.1 - 10 mM) decreased the maximum rates of depolarization (INMAX) and repolarization (OUTMAX) of R and N cells in a dose-dependent manner. In R cells, 3 mM procaine in Ringer containing 8 mM Ca and 2 mM Mg, decreased INMAX from 47.8 V/sec to 49 % of control and OUTMAX from 98.2 to 48 % of control. Reversing the Ca/Mg ratio, while maintaining the divalent cation constant, further depressed OUTMAX to 30 % without greatly changing INMAX. The effects of procaine on action potential parameters were enhanced at alkaline pH (8.5) and decreased at acid pH (6.5). In alkaline pH and/or low Ca (0.2 mM) solution procaine prolonged the R cell action potential from about 3 msec to about 100 - 300 msec. In addition, procaine greatly reduced the Ca-dependent hyperpolarization produced in N cells by repetitive activity (Kleinhaus & Yang this meeting).

In contrast to its effects on excitation mechanisms, procaine 3 mM hyperpolarized the R cell by about 12 mV and depolarized the N cell by about the same amount. These changes in membrane potential persisted in the presence of 20 mM Mg which blocks most leech synapses.

These results show that the Na conductance mechanisms of R and N cells do not differ in their responsiveness to procaine, in contrast to their differential sensitivity to TTX. Furthermore, in these leech neurons, as in other excitable membranes, procaine was more effective in its non-ionized form. Its effects on repolarization mechanisms resulted either from a direct block of a conventional voltage-dependent K conductance or from indirect interference by decreasing Ca entry needed to activate it. Interference of Ca entry necessary for repolarization has been postulated to explain some of the effects of barbiturates and physostigmine on the same cells (Kleinhaus and Prichard, J.Pharm. Exp. Therap. 1977, Yang and King, Neurosc. Abstr. 7, 1981).

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66.7 DIVALENT CATIONS AND THE HYPERPOLARIZATION FOLLOWING REPETITIVE ACTIVITY IN LEECH NEURONS. Anna L. Kleinhaus and Jay Yang* (SPON: L.B. Cohen) Dept. of Neur., Yale U. Sch. Medicine, 333 Cedar Street, New Haven, Ct. 06510.

In leech segmental ganglia, the hyperpolarization following trains of action potentials (PAH) in the cells sensitive to touch (T) cells, pressure (P) cells and noxious stimuli (N) cells, was partly due to activation of a Ca-dependent K conductance present in the order N>P>T (Baylor & Nicholls,J. Physiol. 1969, Jansen & Nicholls, J.Physiol. 1973).

We report here that a similar phenomenon can be evoked in Retzius (R) cells by trains of impulses or steady depolarizations, that Ca plays a role in its generation and that the K conductance responsible for PAH in both cells was sensitive to TEA and Ba.

The magnitude and duration of PAH in R cells, like that in N cells, could be varied with train duration and Ca concentration. In R cells, PAH was accompanied by a reversible fall in input resistance of approximately 10% and was generally of lesser amplitude and duration than in N cells. The difference may in part be explained by the observation that during trains the action potential duration increased more in the N than in R cells. In both cell types, when divalent cation concentration was constant, PAH could be evoked when Sr was substituted for Ca, but not when either Mg or Mn were.

In the presence of tetraethylammonium chloride (TEA) or Ba, which blocked repolarization and caused divalent cation-dependent action potentials in R and N cells (Kleinhaus, Pfluegers Arch. 1976, Kleinhaus & Prichard, J.Physiol. 1977), the hyperpolarization normally evoked by repetitive activity was reduced; under these conditions a 2-3 sec depolarization followed the end of a train or steady depolarization. This depolarization was dependent on the external concentration of Ca or Ba. In solutions containing these ions, but not Mg, the depolarization persisted when Na was reduced or tetrodotoxin (TTX) was present.

These observations suggest that in R cells Ca entry is needed for activation of a K conductance underlying PAH and that, as in molluscan neurons, Sr, but not Ba, can partly substitute for Ca.

The results also provide evidence that in leech N and R cells, as in other vertebrate and invertebrate neurons, this Ca-dependent K conductance is sensitive to TEA and Ba. Neither of these appeared to impair the influx of divalent cations.

Pharmacologic separation of the depolarization due to Ca entry from the hyperpolarization resulting from subsequent K conductance increase may provide a useful method for the study of the interaction of other drugs with these conductances.

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66.8 Calcium-dependence of a component of transient outward current in bullfrog ganglion cells. D.A. Brown*. A. Constanti* and P.R. Adams. Dept. Neurobiology, SUNY Stony Brook, N.Y. 11794 (*permanent address, Dept. Pharmacology, School of Pharmacy, London, U.K.)

We have previously reported that voltage clamped bullfrog sympathetic neurons exhibit a transient outward current (I_t) when returned to a relatively depolarized membrane potential after a short conditioning hyperpolarizing (Constanti, Adams, Brown, Fed. Proc. 39, 2072). We suggested that I_t was an A-current, but now new data suggest that it may be a mixture of A current and transient calcium-activated current. The new data are (1) holding at -20 mV, short steps to -100 mV elicits I_t on returning to -20 mV which is partially blocked by omission of calcium from the Ringer or addition of cadmium. I_t at a holding potential of -40 is calcium insensitive, when present. (2) the calcium sensitive I_t peaks very rapidly (3 to 4 msec) and inactivates more rapidly, than the calcium insensitive component (∿ 10 msec v. 30-50 msec). (3) much shorter hyperpolarizing pulses are needed to remove inactivation of the calcium sensitive component than are needed for the Ca-insensitive component of I_t. Thus only 10 msec pulses remove most of the Ca-sensitive I_t inactivation, whereas recovery of the Ca-insensitive I_t inactivation has a time constant of around 200 msec. Thus we suggest that I_t in these cells is composed of a mixture of A-type current (Ca-insensitive) and calcium-activated current, the former activating at slightly more negative potentials than the latter. Inactivation of the Ca-sensitive and insensitive components of I_t appear to be incomplete or complete respectively. Neither component was sensitive to 1 mM 4 AP. It is likely that the transient Ca-sensitive current is identical to the orthodox calcium-activated K-current (I_C) of these cells (Adams et.al. Nature 296, 746), since both are very sensitive to TEA. Since neither I_C activated by voltage steps during intracellular Ca applications nor I_{Ca} itself show any early inactivation, we suggest that the transient current reported here reflects an initial overshoot of Ca levels just under the membrane following natural calcium entry, perhaps reflecting delays in cytoplasmic buffering mechanisms.

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66.9 CYCLIC NUCLEOTIDE MEDIATED MODULATION OF ELECTROPHYSIOLOGICAL PROPERTIES IN PERIPHERAL NERVE. T. L. Seelig* and J. J. Kendig. Dept. of Anesthesia, Stanford Medical School, Stanford, CA 94305.

Previous studies from this laboratory have shown that dibutyryl cyclic adenosine monophosphate (db-cAMP, a lipophyllic analogue of cAMP) increases steady state outward potassium currents in the voltage clamped node of Ranvier, and that phosphodiesterase inhibitors mimic this effect. In order to determine the physiological relevance of this phenomenon, we examined the effect of db-cAMP (2-4mM) on compound action potential amplitude and refractory period. Refractory period changes were measured as changes in the amplitude of the compound action potential during brief (4-pulse) trains of high frequency stimulation (500-800 Hz). A change in the amplitude of the second response relative to the first indicates a change in refractory period. The studies were carried out at room temperature on the desheathed sciatic nerve of Xenopus laevis, using extracellular killed-end recording.

Thirty minutes after application of db-cAMP, the amplitude of the singly evoked compound action potential had decreased and the refractory period had increased. The effects were reversible on washing with drug-free solution. In parallel control experiments, these properties remained stable following repeated changes of drug-free solution.

Similar changes in amplitude and refractory period occurred after application of a phosphodiesterase inhibitor, SQ20,009 (.05mm) or a beta-adrenergic agonist, isoproterenol (20uM).

When TEA (7.5 mM), which blocks voltage dependent potassium channels, was added to the drug solution after the effects had appeared, the electrophysiological properties returned toward control levels. These results indicate that the observed db-cAMP mediated changes depend on availability of voltage dependent potassium channels.

In order to determine whether the enzymes for cAMP metabolism are available within the axon/myelin system, in vitro assays for cAMP were performed in desheathed isolated sciatic nerves of Xenopus laevis. It was found that cAMP levels were increased eightfold following a 10 minute incubation with either the phosphodiesterase inhibitor IBMX (1 mM) or isoproterenol (20 uM), indicating that the necessary enzymes are present.

These data show that cAMP can modulate the electrophysiological response properties of the nerve via a potassium channel dependent mechanism. Furthermore, this isolated axon/myelin system contains a beta-adrenergic receptor-linked adenylate cyclase that may regulate cAMP levels and modulate the electrophysiological properties of the nerve.

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66.10 RESTING POTENTIAL OF A FROG MYELINATED AXON: THE ROLE OF THE INTERNODE. S.Y. Chiu* (SPON: J. Cooper). Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

Experiments were performed on single frog myelinated nerve fibers to examine whether a normal internode generates a voltage source of its own that is capable of interacting electrotonically with the node of Ranvier. The following observations were made using the technique of Dodge and Frankenhaeuser. (a) A node which was depolarized by an isotonic KCl solution, underwent an abrupt repolarization of 20-30 mV when 3-6 mM TEA was applied externally to the potassium solution. (Tasaki, I., J. Physiol., 148: 306-331, 1959). Substituting isethionate for the external chloride ion did not affect this action of TEA significantly. Prolonged exposure of a node to KCl rendered TEA less effective in causing the repolarization. (b) A Ringer solution containing 60mM TEA depolarized the node instantly by 5-10mV, which was followed by a second slow depolarization of 10-20 mV lasting 10-20 minutes. (c) In response to an abrupt increase in external K concentration, the reversal potentials for the leakage channels E_L and nodal potassium channels E_K reached a similar steady value but with markedly different rates. The response of E_K was immediate, and is consistent with the view that the nodal surface is freely accessible to external ions. In contrast, E_L responded in two phases: there was an initial fast one followed by a second slower one which reached an apparent equilibrium in 20-30 minutes. Thus, part of the resting leakage conductance which determines the resting potential of a node appears to arise from a non-nodal compartment, possibly the internode, with a restricted ionic access. (d) Acute internodal demyelination with lysolecithin revealed a steady resting potential of around -60 mV shortly before a marked reduction occurred in the membrane resistance signalling the destruction of the internodal axolemma. One hypothesis consistent with these observations is that the internodal axolemma normally generates a resting potential underneath the myelin. This hidden internodal battery, though having restricted ionic access, is nevertheless coupled electrotonically to the nodal membrane. Thus, the nodal depolarization induced by potassium is antagonized by this internodal battery, which produces a marked repolarization when the nodal membrane resistance is suddenly increased by TEA. This hidden internodal resting potential, which could be determined by both the internodal K channels and leakage channels, might play a role in controlling the overall resting potential of a normal myelinated nerve fiber.

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66.11 APPLICATION OF THE INDEPENDENCE RELATIONSHIP AND THE GOLDMAN-HODGKIN-KATZ (GHK) CURRENT EQUATION TO CURRENT-VOLTAGE (IV) DATA. Charles L. Bowman* (SPON: Henry Tedeschi). Dept. Biological Sciences SUNY-Albany, Albany, NY 12222.

An important goal in biology is to uniquely describe the mechanisms governing the movement of ions through membranes. One method used to study ionic permeation involves the collection of current-voltage data. I compare two methods that can be used to analyze the data: the independence relationship and the GHK current equation (see B. Hille 'Ionic Selectivity of Na and K Channels of Nerve Membrane' In: Membranes 3: 255 (1975), Marcel Dekker, NY). Both equations can be used to analyze the effect of changing the concentration of permeable ions on the IV curve. For example, the independence relationship can be used to predict another IV curve based on information in the reference IV curve (see above reference). However, the choice of the reference IV curve is arbitrary and large deviations can exist between the predicted and observed IV curves at membrane potentials near the reversal potential of the reference IV curve. It is difficult to decide if the deviations are based on failures of the assumptions used by the independence relationship.

A least squares method and the GHK current equation have been applied to the analysis of experimental data (C. Bowman and A. Baglioni J. Cell Biol. 91: 259a (1981)) to calculate a permeability coefficient, its standard deviation and a theoretical IV curve. The advantage of this method over the independence relationship is that each IV curve can be analyzed separately without the necessity of chosing a reference IV curve. In addition, it is possible to compare the calculated permeability coefficients to determine if a family of IV curves can be uniquely described by a single permeability coefficient. The disadvantages of this approach are that both the surface area over which the current flows and the internal concentration of permeant ions must be known (and be constant). In addition, if the GHK current equation does not describe the data, it is difficult to determine which assumption (or combination of assumptions) is not valid. The assumptions used by the independence relationship and the GHK current equation have been extensively discussed by B. Hille (see reference above) and by R.I. Macey ('Mathematical Models of Membrane Transport Processes' In: Membrane Physiology, T.E. Andreoli, J.F. Hoffman and D.D. Fanestil, editors, Plenum Press, NY P. 125, (1978)).

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66.12 COMPARISON OF ELECTRICAL PROPERTIES OF NERVE MEMBRANE TO THOSE OF PROTEINOID-LECITHIN SPHERES. Wilford P. Stratten* (SPON. Sidney W. Fox). Rose-Hulman Inst. of Tech., Terre Haute, IN 47803.

Artificial cells formed by hydration of thermal proteinoid and lecithin have been reported to manifest electrical characteristics resembling those of natural excitable cells (Ishima, Przybylski, and Fox, BioSystems 13 243-251; 1981). Evaluation of these similarities is enhanced by quantitative comparison of specific electrical components including potential (E), and conductance (g) of the membrane at rest and during transmembrane current clamping, spiking activity, and spike recovery.

The Hodgkin-Huxley Parallel Conductance Model when applied to artificial spherules provides g profiles for the various membrane states. Evaluation of E and g with intrasphere electrodes and current clamping with varying external potassium ion (K) concentrations clearly indicate a significant contribution of g(K). Given g(K), application of the Parallel Conductance equation indicates a significant contribution of sodium, g(Na), to the resting E. The g(Na) may even exceed g(K) if the as yet undetermined contribution of hydrogen (H) and chloride (Cl) ions is significant. An induced reduction in E is accompanied by an increase in g(K) as is the case with nerve membrane, but it also may be accompanied by a decrease in g(Na) which is not manifest with nerve membrane.

The spike depolarization is due to a transient change in g(Na). Given g(Na) as the only contributing conductance change, the increase is small (9 to 15%), but when considering the normal non-spike shifts in g(Na) and g(K) during depolarization, and the probable contribution of Cl and H, the spike g(Na) must be considerably higher; furthermore, the 2 msec duration of this g(Na) transient matches the g(Na) shift of the squid giant axon spike.

Evaluation of the RC constants of the spike recovery phase and the return to resting E upon current clamp cessation indicate complete recovery of the prespike g. The repolarization is due to non-sodium conductance, with g(K) being the most likely contributor.

The protein-lipid structure seems to facilitate not only selective permeability, but also a stereodynamics which allows transient electrical activity of a selective nature. Indication is that selective ion "gates" may not be structures attained by biological systems through eons of natural selection, but rather functional molecular configurations which form due to the nonrandom nature of molecular interaction in nonbiological milieu.

66.13 MEMBRANES WITH TWO STABLE POTENTIALS CAN PROPAGATE SWITCHING WAVES. J. Rinzel. Math. Research Br., NIADDK, NIH, Bethesda, MD 20205.

Under some conditions, excitable membranes may exhibit two different stable steady potential levels V_L and V_U for a range of maintained bias current (e.g. in squid giant axon, see Moore: Nature 183, 1959, 265-266). The membrane potential V may be switched from V_L to V_U and vice versa by appropriate pulses of current. We have examined theoretically such bistable behavior in the context of the full Hodgkin-Huxley model and various Fitz-Hugh type simplifications of this model. Bistability is found for a range of parameter values, e.g. increased extracellular $[K^+]$. Underlying bistability is an N-shaped steady state I-V relation. The N-shape alone however does not guarantee two stable steady states because in some cases only one of the three steady states is stable.

We have explored propagation of signals along a bistable Hodgkin-Huxley cable model. This follows our previous work on a two variable FitzHugh cable (Rinzel & Terman, SIAM J. Appl. Math., in press). For appropriate parameter ranges, a spatially localized stimulus can initiate a V_L to V_U or V_U to V_L switching wave; the two types of waves have different propagation speeds. For some other parameters only one of these waves seems to be possible. Interpretation of these phenomena, which is facilitated by considering certain simplified models, will be presented. The propagation of switching waves may have relevance to signaling by plateau potentials.

68 SYMPOSIUM. USE OF ANIMALS IN RESEARCH. R.K. Dismukes, Chairman, Nat'l. Res. Council; A.L. Caplan*, Hastings Center; T.H.Bullock, UCSD; R. Dubner, NIDR, NIH; R.E. Burke, NINCDS,NIH; H. Edinger, Col. Med. Dent. N.J.; P.J. Hand, Univ. Penn.; C.R. Gallistel, Univ. Penn.

Public concern with treatment of animals in research is rising. Bills have been proposed in Congress and several state legislatures that would restrict, in some cases drastically, the use of animals for research purposes. The debate over animal welfare has been strident, and discussions of ethical, scientific, and public policy issues have often been muddled. It may be argued that neuroscientists have a special responsibility to join the discussion of animal rights because of our special knowledge of the nervous system, perception, and behavior. Furthermore it is clear that failure to respond to public concerns will effect both allocations of research budgets and policies for regulation of research.

Difficult questions are encountered in attempting to develop appropriate guidelines for the use of animals in research. For example, what criteria are appropriate for deciding whether an experimental procedure is acceptable? Do the potential benefits of some experiments more than others justify discomfort and sacrifice of laboratory animals? Should research on some species be more restricted than research on other species, and, if so, what are the criteria? Who should be involved in answering these questions, and how should guidelines be enforced?

A. Caplan will frame ethical issues that need to be considered in the use of animals in research. The use of animals in research is influenced by assumptions about the level of awareness of animals and their experiences of pain. T. Bullock will explore the evolutionary dimension in the question of animal awareness, and R. Dubner will discuss behavioral assessment of pain. R. Burke will examine the problems encountered in attempting to devise guidelines that are rational and specific. H. Edinger will review the legal rules that currently govern use of laboratory animals and he will also discuss recent legislative developments.

After the speakers' presentation, P. Hand and C. Gallistel will lead panel discussion, which will include aspects of veterinary medicine, effects of restrictions on research, and other topics that arise. Questions from the audience will be invited.

69 SYMPOSIUM. PHYSIOLOGICAL INSIGHTS DERIVED FROM NERVE TRAFFIC ANALYSIS IN CONSCIOUS MAN. R. R. Young, Mass. General Hospital, Harvard Medical School (Chairman); A.B. Vallbo*, Univ. of Umeå; E. Torebjörk*, University Hospital Uppsala; B.G. Wallin*, Univ. Hospital,Uppsala; K.-E. Hagbarth*, University Hospital, Uppsala.

A microneurographic method for recording impulses from single myelinated and unmyelinated fibres in peripheral nerves of unanaesthetized human subjects was developed in Sweden about 15 years ago. Vallbo will review peripheral mechanisms involved in tactile sensibility of the hand; 4 kinds of cutaneous units with large diameter nerve fibres may be distinguished. RA and SA I units appear to play a dominant role in spatial analysis of tactile stimuli. Studies of perception in relation to activity in single afferents will be illustrated with findings from psychophysical detection experiments. PC and SA II units may be involved in kinesthesia and position sense—their discharge is closely related to movement and position. Torebjörk-Microneurography can be combined with selective electrical intraneural microstimulation of single identified sensory units. Such activity from regions with particularly rich CNS representation gives rise to sensations which are specific for each type of unit. Qualities and characteristics of such sensations will be described and compared with those of post-ischemic parasthesiae providing new insights into mechanisms of pain and itch. Wallin-In intact man, sympathetic efferents to skin and muscle function differently. Muscle sympathetic activity (pulse-synchronous bursts of vasoconstrictor impulses) counteracts sudden falls in blood pressure but does not set its long-term level. Skin sympathetic activity (vasoconstrictor and sudomotor)affects thermoregulation but also is associated with emotional reactions. Sympathetic outflow is differentiated--the concept of a diffuse "sympathetic tone" can no longer be maintained but with spinal cord transection, sympathetic outflow is similar in skin and muscle nerves suggesting that the differentiation in intact man is due to supraspinal mechanisms. Hagbarth-Single unit recordings from human muscle spindle afferents contradict the theory that voluntary movements and position-holding contractions are generated by a follow-up servo employing spindles. The mean level of Ia firing serves as an afferent "carrier frequency" signalling random, small mechanical disturbances which occur during movement. Resulting dynamic variations in spindle firing play a part in smoothing muscle contraction reflexly. Clonus and Enhanced Physiological Tremor depend on oscillations in the stretch reflex loop but not the resting tremor of Parkinsonism. During voluntary contractions, sudden halts or brisk stretches produce damped intramuscular vibrations (40-60 Hz) which are sensed by the fusimotor-driven spindle afferents. Resulting grouping of spindle discharges produces, monosynaptically, a corresponding grouping of motor discharges. This challenges earlier claims that late EMG peaks in stretch reflex responses are mediated via long-loop transcortical reflex arcs.

70.1 INDUCTION OF NEURONAL BRANCHING IN CAENORHABDITIS ELEGANS. M. Chalfie[1,2*], J. N. Thomson[2*], and J. E. Sulston[2*] (SPON: P. D. Evans) [1]Dept. of Biol. Sci., Columbia University, New York 10027 [2]MRC Laboratory of Molecular Biology, Cambridge, England

The touch cells (microtubule cells) of Caenorhabditis elegans have a very simple structure with a single long process that extends anteriorly from the cell body (the receptor process). The receptor process of all but one of the six touch cells divides at its distal end producing a branch on which a number of important interneuron synapses are made (the synaptic branch). In the ventral cord, the more anterior touch cell (AVM) branches, but the more posterior one (PVM) does not. Both cells, however, are generated by identical cell lineages.

When PVM is generated more anteriorly (by the action of the mutation mab-5), it grows a synaptic branch and makes functionally detectable interneuron synapses. In addition, when AVM is made more posteriorly (by affecting the migration of its precursor by laser microbeam irradiation), it does not grow a synaptic branch and does not form the interneuron synapses. Thus, differences in the location of the cells affect whether or not they branch. Other properties of the cells, however, are not affected by positional differences: ultrastructural features of the receptor process and its length are unchanged.

70.2 DEVELOPMENTAL INDETERMINACY AND HIERARCHICAL ASSIGNMENT OF CELL FATE IN THE LEECH EMBRYO. D. A. Weisblat and S. S. Blair. Molecular Biology, Univ. of Calif., Berkeley, CA 94720.

Segmental ganglia of the leech derive from five bilateral pairs of identifiable blastomeres, the M, N, O, P and Q teloblasts, which themselves arise by stereotyped cleavage of the egg. The teloblasts make smaller blast cells in columns called m, n, o, p and q germinal bandlets; these coalesce ipsilaterally into left and right germinal bands, then longitudinally along the ventral midline as the germinal plate. Labeling individual teloblasts with cell lineage tracers reveals that a blast cell normally generates a segmental complement of progeny that is characteristic of the parent teloblast. Sometimes, however, the pattern associated with the P teloblast is seen when O is labeled, and vice versa. In a similar proportion of embryos, o and p bandlets cross, and are thereby transposed within the germinal band. In fact, it is the relative positions of the bandlets, not the parent teloblasts, that correlates with the progeny distribution patterns. The fates of o and p progeny might still depend on lineage, if one assumes that the O and P teloblasts sometimes exchange places, and that their bandlets then cross and grow into the proper positions within the germinal band. Alternatively, the O and P teloblasts might be of equal developmental potential, and the fates of their progeny determined by environmental factors within the germinal band or germinal plate.

A necessary condition for this second hypothesis to be true is that o and p blast cell progeny be able to assume either "O" or "P" fates. Two types of ablation experiments suggest that this may, in fact, be possible. Firstly, stage 10 embryos in which an O teloblast had been labeled early in stage 7 and the ipsilateral P teloblast was ablated late in stage 7 show a normal "O" pattern anteriorly that abruptly changes posteriorly to resemble the "P" pattern. No such dramatic change occurs in the converse experiment. Secondly, adult leeches raised from embryos in which either an O or a P teloblast had been ablated always show peripheral dopaminergic neurons which arise from P in normal development, but not those from O. This apparent change in fate of blast cell progeny, which we call transmogrification, might reflect a hierarchical assignment of fates to cells of equal developmental potential on the basis of positionally determined interactions.

70.3 POSITIONAL EXPRESSION OF A SURFACE ANTIGEN ON CELLS IN THE NEUROEPITHELIUM AND APPENDAGES OF GRASSHOPPER EMBRYOS. K. Kotrla* and C. S. Goodman. Dept. of Biol. Sci., Stanford Univ., Stanford, CA 94305.

During grasshopper embryogenesis, the ectodermal epithelium gives rise to the neuroepithelium, and the neuronal precursor cells within it then give rise to the neurons. In each segment, 61 neuroblasts (NBs) emerge in 7 rows from a seemingly uniform epithelial sheet. Each NB can be uniquely identified by its position within the sheet, and by the stereotyped identified neurons it generates by a highly invariant cell lineage. We would like to know when and how individual NBs become uniquely determined to generate particular neuronal progeny, and thus have made monoclonal antibodies (mabs) against crude membrane fractions in search of cell surface antigens involved in the process of NB determination.

One of the mabs, Epi-1, stains a cell surface antigen which is positionally expressed on ectodermal epithelial cells throughout embryonic development. In the blastula, Epi-1 stains a broad strip of cells near the midline. In the gastrula, it stains a narrower strip of ectodermal cells near the midline. The staining cells appear no different than their non-staining neighbors; their only distinguishing feature is their position. As the neuroepithelium develops, the Epi-1 staining spreads from the midline to two rows of neuroepithelial cells per segment, including the NBs of rows 2 and 6, which also stain. Other NBs stain later in development. However, none of the neuronal progeny of the NBs stain. Certain epidermal cells at the midline continue to stain throughout development.

The Epi-1 antigen is also positionally expressed on the epidermal cells of the limb buds and other appendages. It stains a broad circumferential stripe of cells near the distal end of each limb bud segment.

Thus, the Epi-1 mab recognizes a cell surface antigen which is positionally expressed throughout development. This antigen is a good candidate for a developmentally important molecule which can convey positional information to neighboring cells. Its positional expression in the neuroepithelium during the formation of the NBs suggests, as one of its developmental roles, a possible involvement in the positional determination of these neuronal precursor cells.

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70.4 CELL LINEAGE AND DETERMINATION OF SEROTONIN AND PROCTOLIN-IMMUNOREACTIVE NEURONS IN GRASSHOPPER EMBRYOS. P.H. Taghert, J.F. Lupatkin and C.S. Goodman. Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305.

In each thoracic and abdominal segment of the grasshopper embryo, 61 neuroblasts (NB's) emerge from the neuroepithelium and each NB generate a family of 10-100 progeny. The resultant 500-3000 neurons in each segmental ganglion show remarkable biochemical and morphological diversity. We are interested in the contributions of mitotic ancestory and cell interactions in the determination of neurotransmitter phenotype in the progeny of specific NB's. In the central nervous system of grasshopper embryos, the number of 5-HT immunoreactive (IR) neurons is small (1%) and the pattern is consistent: conventional immunological controls show the staining to be specific. Immunoreactivity can first be detected at about 55% of development and occurs in axons cell bodies and dendrites simultaneously. The final pattern is reached by 100% of embryonic development.

This pattern is segment-specific: the 6 predominent IR neurons in thoracic ganglia do not appear homologous to the 4 in abdominal ganglia. We have combined 5-HT immuno-staining with Lucifer Yellow injections (and an anti-LY antibody) to identify and trace the lineage of the IR cells in the 4th abdominal segment (A4). The two neurons on either side of the ganglion are sibling interneurons that arise from the fourth cell division of NB 6-5; this NB produces one of the smallest families (12 progeny), all of known morphology. None of these progeny resemble the thoracic IR cells. Many of these progeny persist through the period of embryonic cell death yet they do not become IR.

We have also examined the lineage of two proctolin-IR neurons in A4: both are large, interganglionic interneurons and they arise from the third cell division of NB 4-4. The fourth cell division produces the identified motorneuron, FETi, and the second cell division produces a neuron that is morphologically similar to the proctolin-containing LW cell of cockroaches (O'Shea and Adams, Science 213:567, 1981). Neither of these cells is reliably proctolin-IR.

These results suggest that not all progeny of NB's 6-5 or 4-4 share a common transmitter. However, the normal and invarient lineages that give rise to these 5-HT and proctolin IR cells suggest that mitotic ancestory may, to some extent, influence neurotransmitter phenotype. We are currently testing this hypothesis by laser ablation of specific NB's and progeny.

[Supported by the Muscular Dystrophy Association and the McKnight Foundation]

70.5 INDUCTION OF CHOLINERGIC NEURONAL PROPERTIES IN CULTURED RAT ADRENAL CHROMAFFIN CELLS. A.J. Doupe, P.H. Patterson and S.C. Landis. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

We have grown adrenal chromaffin cells from newborn rats for 3-5 weeks in dissociated cell culture. When cultured in the presence of glucocorticoid hormones, the cells maintain the characteristics of differentiated chromaffin cells: (i) intense formaldehyde-induced catecholamine fluorescence (FIF), (ii) synthesis of large amounts of epinephrine (E) and norepinephrine (NE), and (iii) the presence of numerous large E or NE storage granules in their somas. We have investigated the effects of long-term growth in the presence of nerve growth factor (NGF) and in the absence of corticosteroid on these cultures. Many adrenal medullary cells extend neurites, as previously shown by Unsicker et al. (PNAS 75:3498,1978). Furthermore, these cells lose their intense FIF and their characteristic large chromaffin granules. In addition to growing long processes, these cells contain numerous small synaptic vesicles in axonal varicosities, make synapses on each other, with pre- and post-synaptic specializations, and bind a monoclonal antibody (Chun & Patterson, unpublished) specific for neuronal surfaces. Thus in all respects examined thus far these cells are indistinguishable from mature sympathetic neurons. The transition from chromaffin cell to neuron is being studied at the ultrastructural level. Individual cells with characteristics of both phenotypes are observed after one week in NGF-containing medium.

Chromaffin cells grown in conditions promoting neuronal differentiation can also be induced to acquire cholinergic characteristics by the addition of medium conditioned by heart cells (CM). The cholinergic properties include (i) synthesis and storage of acetylcholine by intact cells, (ii) CAT activity in homogenates, and (iii) the presence of small clear vesicles in many terminals after fixation with potassium permanganate. Thus adrenal chromaffin cells can undergo the conversion from adrenergic to cholinergic phenotype which has previously been demonstrated in sympathetic neurons in vitro and in vivo.

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70.6 ADRENERGIC DIFFERENTIATION OF NEURAL CREST CELLS IN VITRO WITHOUT THE NEURAL TUBE. Marthe J. Howard*, Marianne Bronner-Fraser, and Alisen Tomosky-Sykes* (SPON: I. Chow). Department of Physiology and Biophysics, University of California, Irvine, CA 92717.

The neural crest gives rise to a vast number of derivatives in the embryo, including melanocytes, adrenergic and cholinergic neurons, and supportive cells. Crest cells can be grown in tissue culture according to the method of Cohen (PNAS 74: 2899, 1977); under these in vitro conditions, melanocytes as well as adrenergic cells differentiate readily. The culture medium used in this previous study had high levels of avian embryo extract and horse serum. In the present study, we have examined: 1) if some factor in the embryo extract supports expression of the adrenergic phenotype in the neural crest; and 2) if the presence of the neural tube is a prerequisite for catecholamine production.

Fauquet et al (J. Neurosci. 1 (5): 478, 1981) have proposed that adrenergic differentiation will not occur in vitro when "pure" neural crest cultures (prepared from the posterior neural folds) are explanted without the neural tube. These investigators used a culture medium containing 2% avian embryo extract. In contradistinction, we find that adrenergic differentiation occurs with or without the presence of the neural tube when the embryo extract concentration is increased to 10%. We have demonstrated that catecholamine production (as assayed by formaldehyde-induced fluorescence) can be detected in a wide variety of neural crest explants. These include: 1) neural folds posterior to the last somite of Stage 14 quail embryos; 2) whole neural tubes from Stage 14 quail embryos; 3) dorsal neural tube from Stage 14 quail embryos; and 4) migrating mesencephalic neural crest from Stage 9 quail embryos. All cultures were grown for seven or more days and subsequently assayed for catecholamine histofluorescence. We were unable to detect adrenergic differentiation by catecholamine histofluorescence from any of the above sources of neural crest when the embryo extract concentration was reduced to 2%. These results indicate that there is some diffusible factor in avian embryo extract that must be present in some concentration greater than 2% for adrenergic differentiation to occur. In addition, the results contradict the notion that the neural tube is required for expression of the adrenergic phenotype.

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70.7 ADRENERGIC PHENOTYPE IS INDUCED BY NOTOCHORD IN CHOLINERGIC CELLS IN CULTURE. G. Teitelman, L. Iacovitti, L. Grayson*, T.H. Joh and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

When the cholinergic ciliary ganglion (CG) is transplanted into trunk neural axis, the cells will migrate to the sympathetic chain and express catecholamine (CA) histofluorescence (LeDouarin et al., Proc. Natl. Acad. Sci. 75, 2030, 1978). Since transplanted neurons migrate past the notochord, it is possible that the notochord serves to induce the expression of this CA phenotype in cholinergic cells. To test the hypothesis, we a) developed a system for maintaining notochord in culture, and b) tested whether CG neurons express a CA phenotype when co-cultured with notochord. This was determined by immunocytochemical localization of tyrosine hydroxylase (TH).

Notochords were dissected from the trunk of stage 19-21 chick embryos (Embryonic day (E) 3-3½), treated briefly with trypsin and plated as explants (5-10 mm in length) on collagen-coated tissue culture dishes. Under these conditions, notochords survived for up to 2 weeks and adherent mesenchymal cells proliferated forming a surrounding bed of fibroblasts. An occasional cell with neuronal morphology was observed in these cultures. The day following notochord dissection, CG neurons from stage 32-34 chick embryo (E7-8) were dissociated with trypsin and seeded onto dishes containing notochord. Control cultures contained only CG neurons. Cultures were grown in media as described by Adler et al. (Science, 204, 1434, 1979). This culture system supported the proliferation of ganglionic fibroblasts and the growth and extension of processes from CG neurons for up to 4 weeks.

After 3, 5, 7, or 12 days in vitro, cultures were fixed in 4% paraformaldehyde and stained with specific antibodies to TH according to the PAP method. In nearly 70% of the cultures (12 of 18), CG neurons grown in the presence of notochord contained specific cytoplasmic staining for TH . Staining was seen in neurons both proximal to and at a distance from the notochord. This effect was observed as early as 5 days in vitro. In contrast, control cultures did not stain. Likewise, TH immunoreactivity was never seen in cells of the notochord or in accompanying fibroblasts.

We conclude that notochord induces an adrenergic phenotype in cholinergic CG neurons. Since contact with notochord is apparently not required for the appearance of TH, diffusable factor(s) may be responsible for this induction. These findings suggest that during normal embryonic development, factors released by the notochord promote the expression of a CA phenotype in neural crest cells.
(Supported by NIH Grants HL 18974 and NS 03346.)

70.8 EMBRYONIC DEVELOPMENT OF RAT ADRENAL MEDULLA IN VITRO AND IN TRANSPLANTS TO THE ANTERIOR CHAMBER OF THE EYE. K. Unsicker, T.J. Millar*, H.-D. Hofmann* and T.-H. Müller*. Dept. of Anatomy and Cell Biology, Philipps University, D-3550 Marburg, F.R.G.

The morphological development of the fetal rat adrenal medulla, its response to NGF, dexamethasone (DEX) and anti-NGF antibodies, and the capacity ot prenatal adrenal medullary cells to express a neuronal versus an endocrine phenotype were studied in cultures and in transplants to the maternal anterior chamber of the eye. Organs were taken from stages E 15 through E 21 and explants were cultured in a fully defined medium in Maximov chambers for 4 or 7 days. Maturation was monitored by comparing the ultramorphology of cultured cells with the corresponding in vivo stage. Of all drugs tested, only DEX (10^{-9}M) induced some morphological maturation as evidenced by the appearance of epinephrine-storing granules and an increase in the number and size of catecholamine storage vesicles. Adrenal explants except those taken at E 15 showed spontaneous outgrowth of neurites that could not be prevented by addition of anti-NGF antibodies or DEX. NGF accelerated neurite outgrowth during the initial culture period, but had no clearly demonstrable net effect after 4 and 7 days. In contrast to previous results obtained with postnatal adrenal medullary cells, neurites preferentially grew out from morphologically immature cells, which displayed strong staining for acetylcholinesterase.

Eye chamber transplants showed similar growth and maturation characteristics.

These results suggest that the fetal adrenal medulla requires other or additional factors than those applied and those provided by the cultured adrenal gland in order to fully mature in vitro.

70.9 TRANSPLANTATION OF CULTURED DISSOCIATED MOUSE NEOPALLIUM CELLS INTO THE CNS: A METHOD TO STUDY NEURAL CELL LINEAGES AND DIFFERENTIATION. L.C. Doering* and S. Fedoroff. Department of Anatomy, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0.

During early embryonic development of the CNS many neural cells have similar morphology, especially the proliferative, undifferentiated neural precursor cells. These cells can be isolated at various stages of development and studied in tissue culture. However, we do not know whether tissue culture conditions are always optimal or whether the cells express all their developmental potentials in vitro. Therefore, we have identified cells in culture and transplanted these cells into the brains of neonatal mice. This allows us to determine the developmental potential(s) of the precursor cells and to evaluate the suitability of tissue culture conditions for differentiation of specific precursor cell types.

In cultures of dissociated E15 mouse neopallium we can identify two major cell types: small refractile (SR) cells which form small, partially differentiated neurons and epithelial-like (EL) cells which form astrocytes that contain GFAP intermediate filaments.

When we separate SR cells from EL cells by density gradient centrifugation and transplant SR cells into the cerebellums of neonatal mice, they form neurons (identified by cresyl violet and silver impregnation) which correspond to pyramidal cells and interneurons of the cerebral cortex (analyzed by morphometric nuclear measurements). Such cells are not obtained in vitro indicating that the tissue culture conditions are not optimal for SR cell differentiation. On the other hand, EL cells from 7 day or older E15 neopallium cultures give rise to GFAP positive astrocytes after transplantation. These astrocytes are similar to astrocytes which differentiate in vitro (GFAP staining patterns, morphometric nuclear measurements). This means that although the culture conditions are inadequate for supporting the complete differentiation of neurons, conditions are adequate for astrocyte differentiation.

Using tissue culture in conjunction with transplantation it is possible to determine the developmental potential(s) of neural precursor cells from sequential developmental stages, and observe the end cells they form. It should now be feasible to define the precise temporal relationships of cells in various lineages throughout neurogenesis.

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70.10 SEPARATION OF SUBSETS OF POSTNATALLY DEVELOPING RAT CEREBELLAR CELLS BY ISOELECTRIC FOCUSING. S.V.Kalokhe*[1], N.R.Vaidya*[2], B.P.Gothoskar*[2] and M.G.Deo*[1]. (SPON: W. J. Weiner) 1 Cell and Developmental Pathology Div. and 2 Biological Chemistry Div., Cancer Res. Inst., Tata Memorial Center, Parel, Bombay 400 012. INDIA.

Using a pH gradient generated by Good's biological buffer system in Isoelectric focusing (IEF) technique, the cell populations obtained from rat postnatal cerebellum at 0, 10 and 21 days (P_0, P_{10} and P_{21}) of development were resolved into several subsets differing in their net surface charge. Cells focusing at pI value of 4.9 formed the major fraction in all the three populations studied. The other fractions with pI values 4.45, 4.1, 3.95 and 3.8 were seen to diminish significantly with maturation. Propidium iodide staining of cells in these subsets showed accumulation of tetraploid cells in two subsets of P_{10} population with pI values of 4.45 and 4.1.

Removal of Concanavalin A (Con A)-agglutinable cells from P_0 population decreased the major subset (pI = 4.9) to 50% and other subsets to a significantly greater extent, indicating that 50% of cells in the major subset have receptors for Con A, while all the other four subsets have markedly greater receptors for Con A. Other lectins studied were Wheat Germ Agglutinin and Lens culinaris A lectin.

The results indicate the usefulness of IEF cell separation for following cell surface changes that occur in a differentiating heterogeneous cell population.

70.11 DIFFERENTIATION OF A PARASYMPATHETIC GANGLION. R. David Heathcote, Dept of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305

The peripheral nervous system of vertebrates is derived from the embryonic neural crest. Pluripotential crest cells undergo an extensive migration to their target sites where they differentiate into sensory, sympathetic and parasympathetic neurons and other cell types. This paper examines the initial differentiation of parasympathetic neurons in the cardiac ganglion of the frog, Xenopus laevis.

The "birthdate" of cardiac ganglion cells was determined by ^{3}H-thymidine autoradiography. The earliest time that neurons were observed to undergo their final cell division was around the stage of hatching (Nieuwkoop and Faber stage 33/34). The neurons continue to arise well into metamorphosis. This prolonged period of cell proliferation is unlike other crest derived neurons in sensory and sympathetic ganglia.

Although cardiac ganglion cells are born at hatching, they cannot be identified at their target until a later time. A small number of cells (1 to 3) at the venous end of the heart are revealed at stages 41 to 44 by acetylcholinesterase (AChE) staining. At progressively later times in development, more cells at the venous end of the heart stain for AChE. In addition, these cells have axons that begin to extend throughout the heart. At even later times, large numbers of neurons are added to the ganglion. These cells are distributed throughout the atrium, and do not remain concentrated at the venous end of the heart.

To determine whether the cardiac ganglion precursor cells are born en route to or actually in the heart, a culture technique was devised. Donor hearts from embryos of various stages were transplanted into the peritoneal cavity of host animals. Donor hearts continue beating for months in the host and differentiate to the extent that neurons that previously could not be seen, establish axons that course throughout the transplant. There is not a marked increase in neuronal number, indicating that only a few precursors are present in the transplant; however, those present do become evenly distributed. The extensive axonal network in the transplant is formed and maintained in the absence of the normal presynaptic input.

Thus, cardiac ganglion neurons are born outside the heart and neurons continue to arise over a long period of time.

This study has been supported by a grant from the American Heart Assoc., California Affiliate and an NSF grant to P.B. Sargent.

70.12 EXPRESSION OF A MELANOCYTE PHENOTYPE WITHIN THE HUMAN NEUROBLASTOMA CELL LINE SK-N-SH. Robert A. Ross, Dept. of Biol. Sci., Fordham Univ., Bronx, NY 10458.

The human neuroblastoma cell line SK-N-SH expresses two morphologically distinct cellular phenotypes in culture. One cell type is neuroblast-like with a small rounded cell body and often long neurite-like processes. In contrast, the other cell type is flattened and epithelial-like in appearance. Clones of these two cell types have been isolated and assayed for activities of the catecholamine biosynthetic enzymes tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH). Consistent with the morphological findings, clones with the neuronal phenotype(SH-SY5Y and SH-IN) express activities for both TH and DBH, whereas clones with the epithelial phenotype(SH-EP and SH-FE) do not contain detectable activities for these enzymes. Of interest is the observation that these two cell types undergo bidirectional phenotypic interconversion.

In the present study, I sought to determine whether the epithelial cells represent another phenotype within the repetoire of neural crest cells, from which neuroblastomas arise. Specifically, whether these cells express activity for tyrosinase, an enzyme unique to melanocytes, or 2',3'-cyclic nucleotide phosphohydrolase(CNP), a marker of the glial phenotype. Enzyme activity within the epithelial cells was compared with that in clones expressing the neuronal phenotype.

CNP activity was expressed within both the neuroblast and epithelial-like clones- 11.58 $\pm$ 0.90 μmol/mg/hr in SH-SY5Y and 9.36 $\pm$ 1.02 μmol/mg/hr in SH-EP. These values did not differ significantly from each other, suggesting that the epithelial cell clones of SK-N-SH are not expressing a glial or neurolemmal phenotype.

Tyrosinase activity was detected within the epithelial line SH-FE (0.363 $\pm$ 0.054 nmol/mg/hr). In contrast, activity was undetectable within the neuroblast clone SH-SY5Y. This suggests that the epithelial-like cells of SK-N-SH are expressing a melanocyte phenotype in culture.

The present study shows that neuroblastoma cells, like neural crest cells in vitro, are capable of expressing multiple phenotypes. Since these neuroblast cells can undergo phenotypic interconversion in vitro, they are an excellent model for study of the biochemical regulation of neuronal cytodifferentiation.

70.13 CELL LINEAGE ANALYSIS OF MAMMALIAN CNS DEVELOPMENT: PROGENITOR CELLS ARE COMMITTED EARLY IN DEVELOPMENT IN INDEPENDENT BILATERAL EVENTS. K. Herrup, R. Wetts and A. Letson*. Dept. of Human Genetics, Yale Medical School, New Haven, CT 06510

While the clonal nature of invertebrate nervous development has long been known, it has only recently been demonstrated that cell lineage relationships may play an important role in the development of the mammalian nervous system as well. Quantitative analysis of Purkinje cell number in lurcher chimeric mice reveals the existance of numerical quanta. These quanta are believed to represent the clonal descendents of single Purkinje cell progenitors that are committed to their fate at approximately the neural plate to neural fold stage of development (Wetts and Herrup, Neurosci. Abst. 7, 544 and J. Neurosci., submitted). In C3H/HeJ mice, 8 of these progenitors give rise to the entire Purkinje cell population of each cerebellar half. We report here three new observations on this subject.

First, we have performed a quantitative analysis of the neurons of the facial nucleus of the mouse brain stem. Since there is no known mutant which causes the intrinsic degeneration of facial nucleus neurons, variants of β-glucuronidase activity were used to mark the embryo of origin of the neurons in the chimeras. Five chimeric animals were analyzed in which the histochemical enzyme reaction was successful in an entire set of serial sections through the nucleus. Our analysis suggests that there are twelve progenitors per side that give rise to the facial nucleus neurons. The final size of the facial clones, however, can vary from animal to animal.

Second, we have quantitated the Purkinje cells in the contralateral cerebellar halves from the lurcher chimeras reported previously. We have also examined both left and right facial nuclei from two of our five chimeras. Both of these analyses suggest that the commitment event for each half occurs independently of the event for the other side. This finding is consistent with the fate maps of the neural plate constructed by classical embryological techniques.

Finally, preliminary analysis of lurcher chimeric mice using additional inbred strain combinations suggests that differences in the number of Purkinje cells among different inbred strains of mice can be attributed to differences (presumably of genetic origin) of either the number of clones used to generate the adult number or the size of the individual clones.

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71.1 RETINO-GENICULO-CORTICAL PATHWAYS IN AN ALBINO MONKEY. R.W. Guillery[1], T.L. Hickey[2], J.H. Kaas[3], D.J. Felleman[3] and C.J. Witkop Jr.[4]. [1]Department of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637; [2]School of Optometry, The Medical Center, University of Alabama, Birmingham, AL 35294; [3]Department of Psychology, Vanderbilt University, Nashville, TN 37240; and [4]School of Dentistry, University of Minnesota, Minneapolis, MN 55455.

We have studied the central visual pathways of an adult female albino monkey (Cercopithecus aethiops). She was tyrosinase negative (Witkop et al., Am. J. Hum. Gen., 22:55, 1970), had a white coat and pink eyes, showed a horizontal nystagmus and was esotropic. No evidence for melanin pigmentation in the retina was found ophthalmoscopically or histologically. ^{3}H proline (5mCi in 0.05ml saline) was injected into one eye and the visual cortex was studied electrophysiologically (Kaas and Guillery, Brain Res., 59:61, 1973) 14 days later. Subsequently the brain was prepared for autoradiography. Comparisons were made with normal macaques, with a normal vervet monkey and with one infant albino vervet monkey (Gross and Hickey, Brain Res., 190:321, 1980). As expected, there is a reduced uncrossed retinofugal pathway and an increased crossed pathway. The area of the optic tract occupied by uncrossed fibers is abnormally small as is the uncrossed input to the superior colliculus and the dorsal lateral geniculate nucleus (LGN). There are abnormal laminar fusions in the LGN and, although the distinction between magno- and parvocellular layers is less clear than normal (Gross and Hickey, 1980, loc. cit.), one can see that the abnormal crossed fibers go mainly to the magnocellular layers and to the caudal parts of the parvocellular layers (representing central vision). In the electrophysiological records the normal crossed input to cortex dominated; the abnormal crossed input was weaker and mapped areas near central vision with a normal retinotopic order, but since it was in the wrong hemisphere it formed a mirror reversal of the visuotopic order. That is, the projection resembled the Midwestern rather than the Boston pattern of Siamese cats. Within area 17, the electrophysiological exploration showed that the normal and abnormal afferents were clearly segregated from each other in separate patches or columns. The autoradiographs show crossed transneuronal label as an uninterrupted band in much of area 17, especially on the dorsolateral surface of the hemisphere, where no ipsilateral label was seen. Bands of reduced label are seen in deeper cortex contralaterally and in these regions the ipsilateral cortex shows well labelled patches. Only small areas of cortex show evidence for regular alternating ocular dominance columns. The crossed label generally dominates over the uncrossed label.

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71.2 PROJECTIONS OF INDIVIDUAL LATERAL GENICULATE LAYERS TO THE STRIATE CORTEX IN THE TREE SHREW (TUPAIA GLIS). M. Conley*, D. Fitzpatrick* and I.T. Diamond. Dept. of Psychology, Duke University, Durham, NC 27706.

Of the six lateral geniculate layers in Tupaia, only two, layers 1 and 5, receive fibers from the ipsilateral eye. A variety of evidence (Golgi, cytoarchitectonic, physiological) suggests that ipsilateral layer 1 might be matched with contralateral layer 2 and ipsilateral layer 5 might be matched with contralateral layer 4. Additional support for the idea of matched LGN layers would be forthcoming if it could be shown that both layers in a pair project to the same layer of striate cortex. The questions we tried to answer in this study were: "Does a pair of LGN layers, 1 and 2, or 4 and 5, project to the same cortical layer?" and, "What are the cortical targets of the unmatched layers, 3 and 6?" Two methods were used: 1) tracing the projections of individual LGN layers using the anterograde tracer wheatgerm agglutinin-HRP, and 2) making small injections of HRP into single layers of the striate cortex and identifying the geniculate layers of the retrogradely labeled cells.

The results show that LGN layers 1 and 2, and 4 and 5 are indeed matched inasmuch as the projections of the first pair terminate in cortical layer IVa, and the projections of the second in cortical layer IVb; but while both pairs terminate in the same cortical layer, the pattern of projections from each layer of a pair is unique. Thus, the terminals from LGN layer 1 are more concentrated in the dorsal part of cortical layer IVa than those of LGN layer 2. Similarly, the terminals from LGN layer 5 are more concentrated in the ventral part of layer IVb than those of LGN layer 4. The projections of the unmatched layers are quite different: LGN layer 3 projects to cortical layer I, but more intensely to cortical layer IIIb; LGN layer 6 projects to a thin strip at the base of cortical layer IIIc.

The significance of these results depends upon what (besides one eye from the other) is being segregated by the laminar distribution of cells in the LGN of Tupaia and how this segregation relates to that accomplished by the lamination in LGN of primates and other species. Whether the segregation in LGN of Tupaia is similar or different from that found in other species, the main point is that whatever is segregated in the layers of the LGN remains segregated in the striate cortex.

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71.3 PERCENTAGE OF INTERNEURONS IN THE CAT'S LATERAL GENICULATE NUCLEUS. A.J. Weber* and R.E. Kalil (SPON: P.B. Schechter). Neurosciences Training Program and Department of Ophthalmology, University of Wisconsin, Madison, WI 53706

Present estimates of the percentage of interneurons in the dorsal lateral geniculate nucleus (LGN) of the cat range from less than 10% to greater than 20%. Since interneurons may play an important role in LGN circuitry, it is important to estimate their number accurately. We have therefore attempted to resolve the current discrepancy by making very large injections of horseradish peroxidase (HRP) into visual cortex in order to label geniculate relay cells. We considered all unlabelled nerve cells to be probable interneurons.

Injections of 50-70% HRP, alone or in combination with 2% dimethlylsulfoxide (DMSO) or 2% lysolecithin were made into cortical areas 17 and 18 in 8 cats that ranged in age from 4 wks. to adult. Following survival periods of 48 hours to 6 days, the cats were perfused as follows: 6 with 4% glutaraldehyde in 0.1 M cacodylate buffer, one with 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate buffer, and one with the same aldehyde mixture, but in 0.1 M phosphate buffer. The LGN ipsilateral to the HRP injection was sectioned coronally at 100μ with a Vibratome. The presence of HRP in these sections was demonstrated histochemically by reacting them with diaminobenzidine alone, or after pretreatment with cobalt, nickel cobalt, or cobalt-glucose oxidase. Sections containing heavy labelling were embedded in epon-araldite. One micron sections were then cut from the most intensely labelled region of lamina A and stained with methylene blue and azure II. To determine the percentage of unlabelled cells in each of the cats, all labelled and unlabelled neurons sectioned through the nucleus were counted with a 100X lens. We evaluated the density of cell labelling in two 8 week cats by counting the number of HRP granules in the cytoplasm of 325 labelled cells. In these two animals we also measured the cross sectional areas of all neurons that were cut in the plane of the nucleolus.

Our results indicate that approximately 22% of the neurons in lamina A of the cat LGN remain unlabelled following injections of HRP into areas 17 and 18. Unlabelled cells are the smallest in the LGN, overlapping only slightly the lower end of the labelled cell distribution. Furthermore, we found remarkably little variation in the percentage of unlabelled cells (range 19-26%, mean 22.5± 0.5 S.E.) despite differences in the ages of the cats, fixatives, and HRP protocols that were followed. Similarly cytoplasmic HRP density was not influenced significantly by the addition of DMSO or lysolecithin, phosphate or cacodylate buffer, or any of the HRP methods that we used.

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71.4 SYNAPTIC ORGANIZATION OF THE DORSAL LATERAL GENICULATE COMPLEX IN THE TURTLE, PSEUDEMYS SCRIPTA. P.S. Ulinski. Dept. Anatomy, Univ. Chicago, Chicago, IL 60637.

The dorsal lateral geniculate complex is situated in the dorsal thalamus medial to the optic tract. Neurons whose somata form a plate of cells at the medial face of the complex have dendrites that extend into a neuropile zone situated between the cell plate and optic tract. Neurons with long dendrites parallel to the optic tract are scattered in the neuropile zone. Retinal ganglion cells project bilaterally and topographically to the outer half of the neuropile. Neurons in the geniculate complex project to the visual cortex. The cortex has a reciprocal projection, back to the geniculate complex, that terminates in the inner half of the neuropile and in the cell plate.

Somata of neurons in the cell plate receive few synaptic contacts, although a greater number of synapses involving clear round synaptic vesicles and asymmetric active zones do occur on dendrites within the cell plate. Dendrites of cell plate neurons are occasionally postsynaptic to axon terminals that contain clear round vesicles and participate in asymmetric active zones as the dendrites extend into the inner half of the neuropile. By contrast, the outer half of the neuropile contains a large number of synaptic contacts. Some are on isolated dendrites of medium or small size. However, many synapses occur within glomeruli that are scattered in the outer half of the neuropile. These consist of axon terminals that contain clear round synaptic vesicles and form synapses with asymmetric active zones upon dendrites interdigitating with the terminals. The terminals include those of retinal ganglion cell axons. Many of the dendritic profiles contain pleomorphic vesicles and form synapses with symmetric active zones on other dendrites. The dendrodendritic synapses are sometimes reciprocal; serial arrangements between an axon terminal, a presynaptic dendrite and an ordinary dendrite also occur.

Since both the cell plate and neuropile neurons project to the telencephalon, the lateral geniculate complex in turtles apparently lacks neurons that are involved exclusively in local circuitry. However, the dendrodendritic synapses in the glomeruli provide a substrate for interneuronal transactions within the complex. The synaptic morphology suggests that these synapses are inhibitory. It is reasonable to speculate, then, that interactions between the dendrites of cell plate neurons within the glomeruli lead to local inhibitory events while synapses between the dendrites of neuropile cells and cell plate cells lead to more global inhibitory events.

(Supported by PHS Grant NS 12518)

71.5 A COMPARISON OF THE SYNAPTIC ORGANIZATION OF RETINAL TERMINALS IN THE CAT MEDIAL INTERLAMINAR NUCLEUS AND VENTRAL LATERAL GENICULATE NUCLEUS. R. Ranney Mize and Linda H. Horner*, Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.

The physiological cell classes of the medial interlaminar nucleus (MIN) and the ventral lateral geniculate nucleus (VLG) differ dramatically. MIN has a high percentage of Y cells while VLG contains only W cells. We examined the retinal synapses of these two nuclei quantitatively to see whether we could also detect differences in their morphology and synaptic organization. We identified retinal terminals by their characteristic pale mitochondria. The laminar position, cross-sectional area, and perimeter of each terminal were measured using a computer analysis system. We also measured the contact density of each terminal with conventional and vesicle-containing presynaptic dendrites (F2 profiles) and the density of flattened vesicle axon terminals (F1 profiles) in each field.

The retinal synapses of MIN were indistinguishable from those of the A laminae of the dorsal lateral geniculate nucleus. They were large (mean = 2.5 um^2 area), had moderate contact densities (0.15 per um surface area), and were frequently associated with F2 presynaptic dendrites (0.1 per um surface area). They made contacts with large proximal dendrites, distal dendrites, and dendritic spines. The density of flattened vesicle axon terminals (F1) was 0.06 per um^2. Many of the retinal terminals in MIN formed classic retinal glomeruli and were surrounded by conventional and presynaptic dendrites. A few retinal terminals in MIN were smaller and had simpler synaptic relationships.

The synaptic organization of retinal terminals in VLG was quite different. The terminals were small (mean = 0.94 um^2 area), had higher contact densities (0.20 per um surface area), but were almost never associated with presynaptic dendrites (0.01 per um surface area). They made frequent contacts with spines and small dendrites but rarely with large proximal dendrites. The density of flattened vesicle axon terminals (F1) was 0.09 per um^2. No retinal glomerular synapses were found in VLG.

We conclude that most of the retinal axon terminals in MIN are large while those in VLG are small. Their synaptic relationships also differ markedly, since MIN terminals frequently contact presynaptic dendrites while VLG terminals do not. F2 profiles must therefore be related to Y cells, but not to W cells in the lateral geniculate complex. The virtual absence of presynaptic dendrites in VLG suggests that an inhibitory mechanism which is present in MIN is absent in VLG. The high density of flattened vesicle synapses suggests that other forms of inhibition are present in VLG. (Supported by NIH Grant EY-02973).

71.6 FINE STRUCTURE OF THE THALAMIC RETICULAR NUCLEUS IN THE CAT. Linda S. Ide. Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

Analysis of the fine structure of postero-dorsal portions of the thalamic reticular nucleus (TRN) in the cat reveals an organization similar to that previously described for the perigeniculate nucleus (Ide, Soc. Neurosci. Abstr., 7:459, 1981, and J. Comp. Neur., in press). All of the major classes of synaptic terminals seen in the perigeniculate nucleus are also present in TRN, including a class of relatively large synaptic terminal with round vesicles which makes multiple asymmetric synaptic contacts, and a class of smaller terminals with densely packed round synaptic vesicles, which resembles cortico-thalamic axon terminals present in other thalamic nuclei. At least three types of terminal containing flat or polymorphic synaptic vesicles can be distinguished. One of these contains predominantly ovoid vesicles, is postsynaptic to other synaptic terminals, and, in general, resembles profiles of presynaptic dendrites seen in other thalamic nuclei. These terminals participate in serial and occasional triadic synaptic contacts. While one of the other terminals with flattened vesicles resembles a type of axon terminal common in many thalamic nuclei, the third is a type which occurs in significant numbers in the perigeniculate nucleus but only in small numbers in the A laminae of the dorsal lateral geniculate nucleus (and which, so far, has not been described in other thalamic nuclei). This last type of terminal is medium-sized or small, with large polymorphic synaptic vesicles, relatively pale mitochondria, and, typically, some scattered glycogen-like granules.

In the TRN, as in the perigeniculate nucleus, there are no complex arrays of synaptic terminals and dendrites comparable to the synaptic glomeruli of dorsal thalamic relay nuclei. Relative to the perigeniculate nucleus, somewhat fewer axosomatic synaptic contacts are seen in postero-dorsal TRN, but the pattern of contacts, both onto perikarya and onto dendrites and dendritic spines, are similar in the two nuclei. The parallels between the two nuclei support the idea that they are closely related structures. Brief published observations on TRN in the rat (O'Hara et al., 1980; O'Hara & Lieberman, 1981) suggest important similarities to the present findings in the cat.

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71.7 NEUROTRANSMITTER SPECIFICITY FOLLOWS FUNCTIONAL DIVISION BETWEEN DORSAL AND VENTRAL LATERAL GENICULATE NUCLEI. J. Conway, G. Lynch, S. Lammers* and M. Baudry. Dept. of Psychobiology, University of California, Irvine, CA 92717.

In the rat the dorsal (LGD) and ventral (LGV) lateral geniculates both receive direct retinal input but the composition of this input differs. LGD cells are activated predominately by fast and medium conducting fibers (13 and 6 m/sec) whereas slowly conducting retinal fibers (3 m/sec) project mostly to LGV cells (Hale and Sefton, 1978). These, and other physiological differences suggesting a partial separation of X and Y cells (LGD) and W cells (LGV), prompted us to look at uptake of putative transmitters in the two structures.

Two procedures were used. 1) Slices of rat diencephalon containing the geniculate nuclei were incubated with 2 μM (^3H) glutamic acid in oxygenated Krebs' solution at 25° C for 10 min; fixed in 5% glutaraldehyde and processed for autoradiography. Animals had previously undergone surgery to remove one eye or one visual cortex. The uptake of (^3H) gamma amino butyric acid (GABA) was also tested. 2) Pieces of tissue containing only a) the right LGD, b) the left LGD, c) the right superior colliculus (SC) and d) the left SC were microdissected from 400 μM slices cut on a tissue chopper and homogenized. 100 nM (^3H) glutamate or (^3H) GABA were added to homogenates incubated in 37° C Krebs'. Animals had previously had one eye removed or one visual cortex ablated. Samples were filtered under vacuum and radioactivity measured by conventional techniques and expressed per unit protein.

We found that LGD accumulates glutamate to a greater degree than neighboring thalamic nuclei. The density of uptake on the autoradiograms was noticeably reduced contralateral to enucleation. A reduction of uptake was also found after ipsilateral visual cortex ablation. In LGV uptake of glutamate was not above that of other thalamic nuclei. However, when slices were incubated in (^3H) GABA, the result was that GABA accumulated heavily in the LGV. GABA was taken up to a much greater degree in LGV than in LGD or most other thalamic regions. This uptake was significantly reduced after contralateral eye enucleation. The autoradiographic results closely paralleled the data from the second procedure, uptake of glutamate in LGD homogenates.

These neurochemical results suggest that the LGD and LGV may be utilizing different neurotransmitters. A plausible hypothesis is that the faster conducting system of the LGD uses glutamate as a transmitter and the slowly conducting system in LGV uses GABA. Although GABA is not an excitatory neurotransmitter, its mode of action may not be incompatible with the sluggish W cell response. (Supported by NRSA fellowship IF32NS06942).

71.8 GAD IMMUNOREACTIVE NEURONS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT AND GALAGO. D. Fitzpatrick*, G.R. Penny, D. Schmechel* and I.T. Diamond. Depts. of Psychology and Neurology, and Neurobiology Program, Duke University, Durham, NC 27706.

This report is part of our larger inquiry into the comparison of cell types and their distribution in the lateral geniculate body of different species. In the past, we have used cell size and connections as the basis for identifying classes of neurons. In this study we use the presence of glutamic acid decarboxylase (GAD) immunoreactivity as demonstrated by PAP and avidin-biotin methods to identify geniculate neurons which presumably use the inhibitory neurotransmitter GABA. In both the cat and prosimian Galago, GAD positive cells are found in each geniculate layer, and our samples indicate that these cells comprise 25-30% of the neurons in a layer. The geniculate layers are also characterized by a heavy distribution of GAD terminals and are separated by interlaminar zones which have few GAD terminals. In all layers, GAD positive neurons are significantly smaller than unlabeled Nissl stained neurons in the same region. For example, in layer A of the cat, a sample of GAD cells has a mean soma area of 142 um^2 and unlabeled neurons in the same area have a mean size of 273 um^2. In the magnocellular layers (1 and 2) of the Galago the average size of GAD neurons is 101 um^2, and the average size of unlabeled neurons is 222 um^2. In some cases the proximal portions of the dendrites have been labeled as well as the soma and this allowed us to determine the number as well as the orientation of the primary dendrites. The GAD positive cells display few primary dendrites (2-4). The dendrites on GAD positive cells in the A layers of the cat and the parvocellular layers (3 and 6) of the Galago are mostly vertically oriented, while dendrites of cells in the parvocellular C layers of the cat and layers 4 and 5 of the Galago are mostly horizontally oriented. In addition to GAD positive neurons within the lateral geniculate body, most of the cells within the perigeniculate nucleus of the cat are GAD positive. While there are obvious differences in the laminar organization of the lateral geniculate body of the cat and the Galago, the present results suggest that GABAergic inhibitory neurons constitute a similar class of neurons in both species. Whether GAD positive neurons are, in fact, local circuit neurons is currently being tested by combining retrograde labeling with immunocytochemistry.

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71.9 GABAergic NEURONS AND SYNAPSES IN MONKEY DORSAL LATERAL GENICULATE: A LIGHT AND ELECTRON MICROSCOPIC IMMUNOHISTOCHEMICAL ANALYSIS. M.P. Ogren, A.E. Hendrickson, J. Vaughn, R.P. Barber* and J.-Y. Wu. Dept. of Ophthal., Univ. Wash., Seattle, WA 98195, City of Hope Res. Inst., Duarte, CA 91010, and Dept. Cell Biol., Baylor Med. Sch., Houston, TX 77030.

The morphology and distribution of neurons and synapses in the monkey dorsal lateral geniculate (dLG) that contain the neurotransmitter, gamma amino butyric acid (GABA), were studied by immunohistochemical staining using an antiserum to the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD). The dLG from 6 normal Macaca monkeys were prepared by the peroxidase-anti-peroxidase method for light and electron (EM) microscopy.

Light microscopic analysis shows that labeling of GAD+ structures is largely confined to the dLG laminae including the S layers and is heaviest in the magnocellular layers. A few GAD+ cell bodies are found in all layers. These are small and characterized by a thin rim of labeled cytoplasm around an unlabeled nucleus. Long, straight labeled dendrites are found in continuity with these labeled cells. GAD+ neuropil occurs throughout the dLG, but is more heavily labeled in the laminae. Neuropil labeling consists of small GAD+ puncta, most of which are scattered throughout the neuropil, but a few are found on the surfaces of labeled and unlabeled cell bodies.

EM analysis of labeled cell bodies shows GAD reactivity on the surfaces of Golgi complex cisternae, mitochondria, and scattered in the cytoplasm. The nucleus is unlabeled and has an invaginated nuclear membrane. Proximal dendrites contain mitochondria, cisternae, microtubules and vesicular structures which have labeling associated with their surfaces. Varicose processes are unlabeled in thin regions that intervene between wider portions that contain the majority of labeled organelles.

GAD+ profiles are frequently both pre- and postsynaptic in the same section, suggesting that at least some of them are presynaptic dendritic terminals. GAD+ terminals contain mitochondria and pleomorphic synaptic vesicles that have GAD+ membranes; these avoid regions of postsynaptic contact and accumulate near synaptic specializations that are usually symmetric. Retinal terminals provide the majority of synaptic input to GAD+ profiles, and frequently both are presynaptic to the same dendrite, forming synaptic triads. GAD+ terminals are presynaptic to dendrites and neuronal cell bodies that may be labeled or unlabeled, but they are not presynaptic to other terminal types or to other GAD+ terminals. GAD+ terminals have been observed postsynaptic to two other unlabeled terminal types, including a profile containing flattened vesicles which makes a symmetric synaptic contact. Supported by Grants EY-01208, NS-12116, and the Dolly Green Scholar fund of RPB, Inc.

71.10 THE ROLE OF GABA-MEDIATED INHIBITION IN THE RESPONSES OF LATERAL GENICULATE NEURONES TO MOVING LINE STIMULI IN THE CAT. T.R.Vidyasagar* (SPON: K. Albus). Dept. of Neurobiology, Max-Planck Institute for Biophysical Chemistry, D-3400 Göttingen, West Germany.

When slowly moving long lines are used as stimuli, the responses of most dorsal lateral geniculate neurones show some dependence on the orientation of the line(Vidyasagar,T.R. and J.V.Urbas, Exp. Brain Res., 46, 157-169). This orientation bias is related to the different degrees of end-inhibition in the optimum and non-optimum orientations. This was further tested by the microiontophoretic application of bicuculline methiodide, which is an antagonist of the inhibitory transmitter GABA. The cells were recorded using tungsten-in-glass microelectrodes which had drug-filled micropipettes glued to them. In all the cells, length-response curves in the optimum and non-optimum orientations revealed a significant reduction or complete abolition of the end-inhibition during iontophoretic application of bicuculline.The orientation bias seen with long moving lines was also abolished in most cells. The small residual orientation sensitivity seen in some cells may either reflect the orientation bias of retinal ganglion cells (Levick,W.R. and T.N.Thibos, Nature, 286, 389) or arise from a direct excitatory cortico-geniculate input. In any case, GABA-mediated intrageniculate inhibition underlies most of the orientation bias of the major input neurones to the visual cortex.

71.11 2-AMINO-4-PHOSPHONOBUTYRIC ACID REVEALS INDEPENDENCE OF ON AND OFF CHANNELS IN RABBIT LATERAL GENICULATE NUCLEUS. A.G. Knapp* and L. Mistler* (SPON: B. Dawson). Dept. of Psychology, M.I.T., Cambridge, MA 02139.

2-amino-4-phosphonobutyric acid (APB) selectively inactivates the on channel in the retina by blocking the light evoked responses of the on bipolar cells (Slaughter and Miller, Science, 211:182, 1981). The effects of retinal APB administration on the properties of lateral geniculate nucleus (LGN) cells have been examined in the cat (Horton, Neurosci. Abstr., 7:24, 1981), and in the monkey (Schiller, ARVO Abstr., 22:11, 1982) showing that the on and off systems do not interact significantly at this level. Our study examined the effects of retinal APB infusion on the rabbit LGN, which, unlike that of the cat or monkey, is a relatively unlaminated structure lacking a segregation of cell types.

Two tubes were inserted into the eye to permit circulation of either a control perfusate or a solution containing 200-500 μM APB. Our initial findings indicate that as in the cat and monkey, the on and off channels remain functionally segregated at the level of the LGN. Infusion of APB reversibly abolished the responses of on-center cells to stimulation of both the receptive field center and surround. Our work has failed to reveal any large or reliable effects on the visually evoked responses of off-center cells; center-surround interactions were also unaffected.

In addition to its anatomical homogeneity, the rabbit LGN differs from that of cat and monkey in containing motion and direction selective cells. We found that APB abolished the light-edge responses of these cells; the dark-edge responses continued to be motion or direction selective. These findings suggest that preference for motion or direction, which in the rabbit arises at the retinal level, does not result from an interaction between the on and off systems. It appears therefore that these two channels have independent access to the mechanism(s) subserving motion and direction selectivity.

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71.12 THE CONSEQUENCES OF 2-AMINO-4-PHOSPHONOBUTYRIC ACID (APB) EYE INJECTIONS IN THE MONKEY AS DETERMINED BY ELECTROPHYSIOLOGICAL AND BEHAVIORAL MEASURES. J.H. Sandell and P.H. Schiller*. Dept. of Psychology, M.I.T., Cambridge, Mass. 02139.

Infusion of 2-amino-4-phosphonobutyric acid (APB) into the eye selectively attenuates the ON responses in the retina, the lateral geniculate nucleus (LGN) and the visual cortex (Schiller, ARVO Abstr., 22: 11, 1982); the OFF responses are largely unaffected with short-term infusions (Slaughter and Miller, Science, 211:182, 1981). This study examines the long-term effects of APB injection into the vitreous chamber of the eye using both electrophysiological and behavioral measures.

Our electrophysiological studies show that injection of 0.1 cc of 8 mM APB into the vitreous of the monkey eye (final estimated concentration in the vitreous: 200 μM) blocks the responses of on-center parvocellular LGN cells within 1-2 hr. Magnocellular on-center cells lose their responsiveness 3-5 hr after the injection. The OFF responses remain largely unaffected for the first 24 hr. After several days, however, OFF responses are also reduced, with parvocellular cells attenuated more than magnocellular cells. Repeated recordings in these animals showed that recovery from APB injection took more than 6 weeks.

The behavioral effects of APB injection were assessed by obtaining measures of contrast sensitivity, brightness discrimination and visuo-motor integration. We used a staircase procedure to determine contrast sensitivity for gratings of several spatial frequencies and brightness discrimination over a 2 log unit range. When tested 3.5-4 hr after injecting one eye with 0.1 cc of 8 mM APB, the minimum contrast that the monkey could detect with that eye was 69% for a 4.25 cpd grating and 8.5% for a 0.5 cpd grating. After an additional 2 hr the contrast threshold for both spatial frequencies was 64%. Subsequent daily testing revealed a progressive loss in the ability to detect gratings and brightness differences. Visuomotor behavior at this point also became severely degraded; picking apple pieces from a slotted board (Schiller, et al., J. Neurophysiol., 44: 1175, 1980) was no longer possible when the monkey was forced to use only the injected eye. Initial signs of recovery did not occur until at least 4-5 weeks after the injection. Performance as measured through the intact eye remained unimpaired throughout, with the minimum detectable contrast consistently less than 5%.

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72.1 DAMAGED PERIPHERAL NERVE AXONS REGENERATE IN A MILIEU OF INCREASED $[K^+]_e$. PHYSIOLOGIC IMPLICATIONS FOR NEUROPATHIC SYMPTOMS. P. A. Low. Neurophysiology Laboratory, Peripheral Nerve Center, Mayo Clinic, Rochester, Minnesota 55905.

In vitro studies have consistently demonstrated potent effects of extracellular potassium $[K^+]_e$ on peripheral nerve membrane potential and excitability. However, direct measurements of endoneurial fluid K^+ has never been reported and the alterations in disease states have not been studied.

The $[K]_e$ was measured with K^+ ion-sensitive microelectrodes in normal, demyelinated and regenerating rat peripheral nerve fibers. The mean values were 3.7 mM (sd 0.7 mM, n = 14) in normal nerve, 4.8 mM (sd 2.9, n=11) in demyelinated nerves and 11.4 (sd 5.7, n = 8) in regenerating nerve fibers. When normal or demyelinated nerves were subjected to electrical stimulation or ischemia, $[K^+]_e$ increases such as those observed in CNS were never seen. The $[K]_e$ was consistently elevated in regenerating nerves and infrequently in demyelinated nerve. Since K^+ is known to increase spontaneous firing in damaged peripheral sensory nerve fibers, the present findings suggest a mechanism in the production of spontaneous firing and positive symptoms in peripheral neuropathies.

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72.2 IMPULSE GENERATORS WITHIN PATHOLOGICAL HUMAN PRIMARY SENSORY NEURONS: ECTOPIC DISCHARGES AND ABNORMAL SENSATIONS. J. Ochoa, E. Torebjörk*, W. Culp* and W. Schady*. Section of Neurology, Dartmouth Medical School, Hanover, NH 03755.

It was shown earlier (Torebjörk et al, Acta Physiol Scand, 105:518, 1979; Ochoa and Torebjörk, Brain 103:235, 1980) that when nerves of otherwise normal human subjects are manoeuvred into transiently evoking spontaneous abnormal sensations, like postischemic paresthesiae, the electrophysiological correlate at the single sensory nerve fiber level are bursts of discharges generated ectopically. Here we report microneurographic evidence of comparable (though spontaneous) behavior in pathological sensory nerves of humans reporting positive sensory symptoms referred to the innervation territory of the nerve sampled.

(1) Mechanosensitive fibers in sensory nerves: the clinical sign of Tinel. Gentle mechanical tapping on a traumatic neuroma elicits abnormal sensations referred distally onto the original nerve territory. Concomitantly, intraneural recording from skin nerve fascicles, proximal to the site of injury and Tinel sign, reveals ascending volleys of impulses with consistently long latencies. The parameters indicate conduction along original small caliber fibers or along immature sprouts, rather than reflex sympathetic activity.

(2) Chronic, spontaneous, ectopic discharges, and paresthesiae from locally injured sensory nerves. Paroxysmal unitary discharges of relatively high impulse frequency, repeating in bursts of fairly regular duration and interval, have been recorded microneurographically on several patients, weeks and months after moderate mechanical injury to the corresponding nerve. At the time of recording, the patients volunteered spontaneous ongoing "tingling" and 'pins and needles' referred to a skin territory inclusive of the projection area of the sensory fascicle being recorded. A clinical sign of Tinel was consistently present too. Unlike normal ongoing cold unit, C-sympathetic or SA-II mechanoreceptor activity, the spontaneous discharges were paroxysmal and usually not influenced by natural afferent or reflex stimuli.

Conclusions. Through microneurographic recording, ectopic impulse activity, either spontaneous or mechanosensitive, and either experimentally induced or symptomatic of pathology, can be documented at the single fiber level from sensory fascicles in awake human subjects experiencing abnormal sensations.

72.3 FINE AFFERENT UNIT DISCHARGE CHARACTERISTICS OF INFLAMED KNEE JOINTS IN THE CAT. R.E. Coggeshall, K.A. Hong*, L.A. Langford, H.-G. Schaible* and R.F. Schmidt. Physiologisches Institut der Universität, D-2300 Kiel, Fed. Rep. of Germany.

An experimental knee joint inflammation was produced by injection of 1.5 ml of carrageneen (2%) and kaolin (2%) or 0.06 mg of TPA dissolved in DMSO and Tyrode into the joint cavity. Five to six hours later when the joint was edematous and the body temperature had risen to 39°C, single unit recordings were started in filaments dissected from the saphenous nerve in the upper thigh. The units were identified as belonging to the medial articular nerve by electrically stimulating this nerve close to the joint capsule. All units could be activated by mechanical stimulation of the medial and anterior joint capsule including the medial collateral and patellar ligaments. Units with conduction velocities between 2.5 m/s and 20 m/s were classified as belonging to Group III, those with conduction velocities less than 2.5 m/s as Group IV. The properties of these units were compared to those sampled from normal knee joints using the same techniques (Kanaka, Schaible, Schmidt, Pflügers Archiv 391: R44, 1981; Neuroscience 7: S109, 1982; Schaible, Schmidt, Wendisch, Pflügers Archiv 392: R46, 1982).

The proportion of units displaying resting discharges was definitely higher in the inflamed group, especially in the Group IV fiber range. The frequency of discharge was also higher and often bursts were superimposed upon background resting activity. The receptive fields were much larger than those in the control units and the thresholds to von Frey stimulation were lower.

During joint movements (flexion, extension, rotation, abduction, adduction) units with receptive fields on the ventral aspect of the joint discharged readily – a pattern never seen in the control animals. Units with receptive fields on the medial aspect also discharged in a higher percentage and with lower thresholds to joint movement than in the control sample.

Finally, it is noteworthy that in the inflamed joint, fine afferent units were seen which differed in no way from those seen in normal joints.

Light microscopic controls of joint capsule tissue taken after the recording session (12-16 hours post induction of inflammation) revealed histopathological signs of inflammation with slight to massive cellular infiltration of edematous tissue.

In conclusion, the experimental inflammation increased the resting and evoked discharges in many but not all fine afferent units in the medial articular nerve. As a result, in both the static and mobile joint, the total afferent outflow transmitted via these fine afferents to the spinal cord is considerably increased.

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72.4 PROLONGED INHIBITION OF THE CAT FLEXION REFLEX BY PERIPHERAL NERVE STIMULATION. J.M. Chung, Z.R. Fang*, C. Cargill* & W.D. Willis. Marine Biomed. Inst., Depts. of Anat. and Physiol. & Biophys., Univ. Texas Med. Br., Galveston TX 77550

A long-lasting inhibition of the flexion reflex was produced by prolonged electrical stimulation of a peripheral nerve with high intensity and low frequency pulses in decerebrate and spinal cats. The flexion reflex in single active motor axons was recorded from filaments of the L7, S1 or S2 ventral roots. The reflex was elicited either by electrical stimulation of a cutaneous or mixed hindlimb nerve or by natural forms of stimulation applied to the foot. A late flexion reflex discharge could be elicited by electrical stimuli that activated Aδ and C afferent fibers. Conditioning stimulation of the common peroneal or tibial nerve at suprathreshold intensity for C fibers at a rate of 2 Hz for 15 or 30 min produced an inhibition of the flexion reflex late discharges which outlasted the conditioning stimuli. Maximum inhibition on average was to 40.1% and 42.7% of control reflex value in decerebrate and spinal cats, respectively. In decerebrate cats, the duration of inhibition varied from less than 10 min to over an hour beyond the termination of the conditioning stimuli, depending on the unit. However, inhibition lasted over 20 min for all units tested in spinal animals. This long lasting inhibition of the flexion reflex was reversed completely by systemic injection of naloxone hydrochloride (0.05 mg/kg). The long-lasting inhibition of the flexion reflex produced by peripheral nerve stimulation will be discussed in relation to peripheral nerve stimulation produced analgesia. (Supported by NIH grants NS 09743 and NS 11255, a fellowship (to Z.R.F.) from the World Health Organization, and a grant from the Moody Foundation).

72.5 RELATIONSHIP BETWEEN LOCUS OF CHORDOTOMY AND TIME COURSE OF HYPOREACTIVITY TO NOXIOUS STIMULATION IN MONKEY. J.D. Greenspan, L.A. Ritz[1] and C.J. Vierck[1,2] [1]Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27514; [2]Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

Eight pig-tailed macaque monkeys were trained to pull a manipulandum in order to terminate electric current applied to either hindlimb. Three intensities were used (0.4, 1.1, and 2.5 mA/mm^2 AC), which range from pain threshold to strong but tolerable pain when applied to human beings under identical conditions (Vierck, Franzen & Cooper, Neurosci. Abstr. 6: 430, 1980). Following a period of behavioral training and preoperative data collection, each monkey received a chordotomy on the right side at the upper to midthoracic level. A week after surgery, we resumed assessment of the monkeys' reactions to electrocutaneous stimulation. After several months of testing, each animal was sacrificed and the locus of the lesion determined.

Immediately following the chordotomy, all monkeys exhibited hyporeactivity, indicative of reduced sensitivity to noxious electrical stimulation of their left leg (contralateral to the lesion). Three monkeys showed little or no indication of recovery of sensory function in the left leg for up to 17 months after the lesion. These three had unilateral lesions of only the right ventrolateral column and the ventral portion of the right ventral funiculus. Three other monkeys exhibited slight to moderate deficits initially, with a rapid and complete recovery (within 2-3 months postoperatively). The lesions of this latter group were complete or almost complete ventral quadrant lesions on the right side, and extended past the midline to involve all or almost all of the ventral funiculus on the left side. The two other monkeys showed moderate deficits initially, with some degree of recovery; however, the recovery was not consistently comparable to preoperative levels. One of these two, showing greater recovery, had a complete ventral quadrant lesion which crossed the midline to involve most of the left ventral funiculus. The other of the two had a complete right ventral quadrant lesion with no left side damage.

These data have been confirmed by a separate series of experiments on Cebus monkeys (Vierck, unpublished observation) and suggest that a ventrolateral chordotomy that is strictly unilateral and spares the dorsomedial portion of the ventral funiculus allows for little or no recovery from sensory loss. In contrast, a ventrolateral chordotomy that encompasses both ventral funiculi is associated with sensory recovery.

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72.6 DISCRIMINATION OF NOXIOUS THERMAL STIMULI IN HUMAN AND MONKEY. M.C. Bushnell, M.B. Taylor*, J. Ziriax*, G.H. Duncan*, and R. Dubner. Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, Maryland 20205.

Nociceptive primary afferents produce graded responses to noxious thermal stimuli of increasing intensity (43°-$51^{\circ}C$). Thermoreceptive primary afferents (warm fibers) produce comparable response gradations to small increases in skin temperature in the 30°-$43^{\circ}C$ range, but very few show monotonic responses to noxious heat stimuli. We looked at human and monkey observers' ability to use this information to make fine discriminations between thermal stimuli applied to the face.

Four human subjects performed a two-choice discrimination task, in which 3-sec heat pulses were presented simultaneously on two contact thermodes positioned bilaterally on the hairy maxillary lip. In some sessions subjects compared $47^{\circ}C$ to stimuli $0.01^{\circ}C$ to $1.0^{\circ}C$ less than $47^{\circ}C$, while in other sessions they compared $39^{\circ}C$ to stimuli $0.01^{\circ}C$ to $1.0^{\circ}C$ less than $39^{\circ}C$. Subjects reported which stimulus was hotter by pressing one of two keys and were immediately notified of correct judgements by a 1-sec tone. Percent correct was determined at each temperature difference (ΔT).

All subjects showed a positive monotonic relationship between ΔT and percent correct for comparison stimuli of $47^{\circ}C$ and $39^{\circ}C$. In addition, for each $\Delta T \geq 0.1$ all subjects produced more accurate discriminations at $47^{\circ}C$ than at $39^{\circ}C$. The difference threshold, defined as the smallest ΔT detected on 75% of the trials, was smaller for every subject at $47^{\circ}C$ than at $39^{\circ}C$, and the mean difference threshold of $0.31^{\circ}C$ at $47^{\circ}C$ was significantly different from the mean difference threshold of $0.54^{\circ}C$ at $39^{\circ}C$ ($t < .05$, $df = 3$).

One rhesus monkey was trained to perform the same two-choice discrimination task, using a $47^{\circ}C$ comparison stimulus. The thermodes were placed bilaterally on the maxillary hairy lip, and the monkey received liquid reward in addition to the tone signal for correct responses. The monkey's performance was indistinguishable from that of the humans. Larger ΔT's led to higher percentages correct, and the difference threshold was $0.28^{\circ}C$.

These data show that humans and monkeys are able to use information provided by nociceptive primary afferents to make precise discriminations between noxious thermal stimuli. Additionally, humans make more accurate discriminations to noxious heat stimuli than to innocuous warming stimuli. This increased discriminative ability in the noxious heat range suggests that there is more secure central nervous system processing of stimulus intensity information arising from thermal nociceptors than from warm fibers.

72.7 NALOXONE AND PLACEBO ALTER POSTSURGICAL PAIN BY INDEPENDENT MECHANISMS. R.H. Gracely*, P.J. Wolskee*, W.R. Deeter* and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

The effect of hidden infusions of naloxone on the change in postsurgical pain produced by placebo, fentanyl or no treatment was assessed in 93 dental patients undergoing oral surgical extraction of a lower third molar under lidocaine local anesthesia without a vasoconstrictor. Ten mg naloxone or naloxone vehicle was administered double-blind two hours after surgery in a hidden infusion through an indwelling intravenous line. This infusion was followed immediately by the double-blind unhidden administration of 0.11 µg/kg fentanyl, saline placebo or no treatment.

Patients completed the Pain Rating Index of the McGill Pain Questionnaire at 60 and 10 min before, and 10 and 60 min after, the drug administrations. Treatment effects were computed using the 10 min before administration measure as a baseline. The Pain Rating Index was sensitive to the effects of the opiate fentanyl and the antagonism of these effects by naloxone. Pain after the combination of hidden naloxone vehicle and fentanyl was significantly less than pain after the combination of hidden naloxone and fentanyl at both 10 min ($t(31)=4.25$, $p < .0005$) and 60 min ($t(31)=2.20$, $p < .05$). The effects of hidden naloxone on placebo and no treatment at 60 min were assessed by a 2-way analysis of covariance (hidden infusion of naloxone or naloxone vehicle X unhidden infusion of placebo or no treatment) with the baseline pain as the covariate to adjust for individual differences in baseline level. Pain was increased significantly after naloxone ($F(1,53)=6.97$, $p < 0.02$) and decreased significantly after placebo ($F(1,53)=10.31$, $p < 0.005$). There was no significant interaction between these effects, strongly suggesting that naloxone and placebo produce effects that are both separate and independent.

These results indicate that naloxone increases surgical pain by mechanisms that may include antagonism of endogenous opioid compounds released due to the stress of surgery. Placebo reduces postsurgical pain by a nonopioid mechanism. The combined action of these separate effects is sufficient to explain the increase in pain in comparison to placebo produced by naloxone as well as its apparent reversal of the placebo effect.

72.8 DOES FENTANYL ANALGESIA INVOLVE THE BRAINSTEM RETICULAR FORMATION? O. Yuge*, L.M. Kitahata, J.G. Collins, M. Suzukawa*, M. Matsumoto*, M. Tabatabai* (SPON: R. LaMotte). Dept. of Anesthesiol., Yale Univ. School of Med., New Haven, CT 06510

The reticular formation has been considered for decades to be an important site of anesthetic action. In more recent years, specific loci within the reticular formation have been identified as playing an important role in the production of analgesia. The nucleus reticularis gigantocellularis (NRGC) located at the caudal end of the reticular formation has been described as a relay center for pain information ascending from the spinal cord to higher brain centers. The present study was carried out in order to determine if the intravenous administration of fentanyl is capable of altering noxiously evoked neuronal activity measured at the level of the nucleus reticularis gigantocellularis.

Extracellular single unit recordings were obtained from NRGC neurons in decerebrate animals. Neurons were activated by supramaximal A-delta electrical stimulation of the superficial radial nerve. Only neurons which responded exclusively to supramaximal A-delta stimulation were studied. The animals had been surgically prepared with femoral artery and vein cannulation and suboccipital craniotomy under halothane, nitrous oxide, oxygen anesthesia, but at least two hours passed between the end of anesthetic administration and the start of drug studies. Physiologic parameters were monitored and maintained within normal limits. Fentanyl, 25 µg/kg, was administered intravenously in a volume of 2 ml of physiologic saline. Spontaneous and stimulus evoked activity was monitored continuously to observe the effects of fentanyl. Thirty minutes after fentanyl administration, naloxone, 0.1 mg, was administered intravenously.

The intravenous injection of 25 µg/kg of fentanyl caused a significant suppression of the mean noxious evoked activity within 10 minutes. Maximum suppression from control values occurred within 15 minutes after fentanyl administration. The intravenous administration of naloxone, 0.1 mg, produced a significant reversal of the fentanyl-produced suppression.

Few studies have evaluated the ability of opioids to suppress noxiously evoked activity as recorded at the level of the NRGC. Fentanyl suppression of activity elicited by supramaximal A-delta (noxious) stimulation supports the concept that opioid analgesia may influence neuronal activity recorded in the caudal reticular formation. Naloxone reversal of this suppression indicates that it involves a specific drug-receptor interaction rather than non-specific suppression of neuronal activity.

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72.9 EFFECTS OF SPINALLY ADMINISTERED FENTANYL ON WIDE DYNAMIC RANGE NEURONS IN THE DORSAL HORN. M. Suzukawa*, M. Matsumoto*, J.G. Collins, L.M. Kitahata. Dept. of Anesthesiol., Yale Univ. School of Med., New Haven, CT 06510

The spinal placement of opioids is a promising new technique in the treatment of pain. Because of the clinical importance of this technique, it is imperative that we obtain a better understanding of its effects and limitations. This study was carried out in order to determine the dose response relationships, time course and potential naloxone reversibility of the effect of the potent, lipid soluble opioid, fentanyl, on noxiously evoked activity of wide dynamic range neurons.

Extracellular, single unit recordings were obtained from physiologically identified, wide dynamic range neurons in decerebrate, spinal cord transected (L-1) cats. The animals had been surgically prepared (tracheal, carotid artery, and jugular vein cannulation and lumbar laminectomy L 4-7) under halothane, nitrous oxide, oxygen anesthesia, but at least 4 hours passed between the end of anesthetic administration and the start of drug studies. Physiologic parameters were monitored and maintained within normal limits. Spontaneous and stimulus evoked activity (radiant heat stimulus of 51^{0}C, for 8 seconds applied to the peripheral receptive field located on the hind paw foot pad) were studied both during the control situation and following the spinal administration of either 10 or 25 µg of fentanyl. Thirty minutes after fentanyl administration, 0.1 mg of naloxone was administered intravenously.

Spinally applied fentanyl suppressed both spontaneous and noxiously evoked activity of all WDR neurons studied. 10 µg produced significant suppression within 6 minutes, whereas 25 µg produced significant suppression within 3 minutes. The 25 µg dose produced significantly greater suppression at all time points than did the 10 µg dose. Within 2 minutes after 0.1 mg of intravenously administered naloxone, there was significant reversal of the suppression produced by both doses of spinally administered fentanyl.

The presence of a dose response relationship in experimental animals suggests that optimal dosages can be determined for the production of analgesia in humans. The very short time course seen in experimental animals compares favorably with time courses seen in humans and thus suggests that interruption of neuronal signals carried by WDR neurons may be important in the production of spinal opiate analgesia. Naloxone reversal substantiates the fact that this effect is due to a specific drug receptor interaction rather than to a non-specific suppression of neuronal activity.

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72.10 EVIDENCE THAT ENDOGENOUS OPIATES CONTRIBUTE TO THE MEDIATION OF VAGINAL STIMULATION-PRODUCED ANTINOCICEPTION IN RATS. J.L. Steinman*, L.A. Roberts*, and B.R. Komisaruk (SPON: J. Moreines). Inst. of Animal Behavior, Rutgers Univ., Newark, NJ 07102.

Previous studies have shown that naloxone HCl (10 mg/kg) attenuates vaginal stimulation-produced antinociception (VSPA) on certain behavioral tests (latency of tail withdrawal from warm water (TWL) or radiant heat (TFL) (Hill and Ayliffe, 1981: Pharmacol. Biochem. Behav. 14, 631) but not others (threshold of vocalization in response to electrical tail shock) (Crowley, Rodriguez-Sierra, and Komisaruk, 1977: Brain Res. 137, 67). In the present study both of these findings were confirmed (n's: 8-10 in each group). TFL: The proportion of rats showing maximum inhibition of 6+ sec during vaginal stimulation (VS) (200g force) was significantly lower in the naloxone (38%) than the saline group (80%) ($p<.05$). TWL: the maximum percent effect during VS was significantly lower in the naloxone (0.60) than the saline (0.88) group ($p<.025$). By contrast, the vocalization threshold during VS did not differ significantly between the two groups. In addition, naloxone attenuated VSPA on a new test - threshold of tail flick in response to electrical tail shock. On this measure, the threshold during VS in the naloxone group was 65% of the saline group ($p<.01$). These findings are further supported in a study demonstrating cross-tolerance between morphine and VSPA. Female rats were rendered tolerant to morphine by twice daily injections of morphine sulfate (20 mg/kg) for 10 days. In morphine-tolerant rats, VSPA was significantly attenuated on the naloxone sensitive TWL test (maximum percent effect: 0.89: saline, 0.51: morphine tolerant; $p<.025$), but not on the naloxone-insensitive test of vocalization threshold to electrical tail shock. These findings suggest that endogenous opiates contribute to the effect of VSPA, suppressing responses mediated at intraspinal (tail flick) but not supraspinal (vocalization) levels. Supported by NSF grant BNS78-24504 (BRK).

72.11 AUTORADIOGRAPHIC LOCALIZATION OF OPIATE, BETA-ADRENERGIC, CHOLINERGIC AND GABA RECEPTORS IN THE MIDBRAIN PERIAQUEDUCTAL GRAY. A.J. Beitz, J. Buggy, L. Terracio*, and W.E. Wells. Depts. of Anatomy and Physiology, Univ. of S. Carolina, Columbia, S.C.29208.

The radiohistochemical technique developed by Young and Kuhar (1979) was employed in the present investigation to analyze the distribution of opiate, muscarinic cholinergic (ACH), beta-adrenergic (ADR) and GABA receptors in the rodent midbrain periaqueductal gray (PAG) and its four anatomical subdivisions. Forty adult rats were perfused with 0.1-0.2% paraformaldehyde. The brains were removed, frozen at -40°C and sectioned on a cryostat. ^{3}H-naloxone and ^{3}H-quinuclidinyl benzilate were utilized to localize opiate and ACH receptors, respectively. ^{3}H-dihydroalprenolol and ^{125}I-pindolol were used to evaluate ADR binding, while ^{3}H-muscimol was utilized to demonstrate GABA receptor localization. Prior to autoradiographic exposure the appropriate incubation parameters and wash times were determined for each ligand using scintillation counting. Resolution of opiate and ACH receptors was enhanced by introducing a liquid fixation step following the incubation and initial wash steps. In addition, specific GABA binding was increased by preincubating the tissue sections in 0.2% triton, prior to the incubation step. Following the incubation procedure, tissue sections were dried and then either apposed to LKB ultrofilm or emulsion coated coverslips, or they were dipped directly in emulsion. The resulting autoradiograms were analyzed by computerized image analysis densitometry or by darkfield microscopy. High levels of opiate receptors were found in the ventrolateral PAG subdivision at inferior collicular midbrain levels but rostrally were concentrated in both the ventrolateral and dorsolateral subdivisions. The dorsal PAG subdivision and a distinct area in the lateral portion of the PAG contained low levels of opiate receptors. A moderate amount of ACH receptors were found in the PAG and these were concentrated in the dorsolateral subdivision and in an area along the dorsal aspect of the cerebral aqueduct (the dorsal half of our medial PAG subdivision). Moderately high levels of ADR receptors were evident throughout the PAG. ADR receptors, however, were most concentrated in the dorsal half of this midbrain region and were not localized to a particular PAG subdivision. Low levels of GABA receptors were found throughout the PAG. This receptor type, however, was more concentrated in the dorsal PAG subdivision and along the lateral aspect of the dorsolateral subdivision. Previous work in this laboratory has demonstrated four intrinsic subdivisions in the PAG using morphological techniques. The present work supports the existence of these subdivisions but in addition suggests that other pharmacological subdivisions are present in the PAG. Supported by NSF BSN7906486 and in part by NIH NS-17401.

72.12 AN ADDITIONAL MECHANISM OF EPINEPHRINE ENHANCEMENT OF SPINAL BLOCKADE BY LOCAL ANESTHETIC AGENTS. J.G. Collins, L.M. Kitahata, M. Matsumoto*, M. Suzukawa*, E. Homma*, Dept. of Anesthesiol., Yale Univ. School of Med., New Haven, CT 06510

It is clinically accepted that the co-administration of epinephrine with local anesthetics enhances spinal block. This effect has been attributed to vascular constriction resulting in slower drug removal. Increasing knowledge of the neurophysiology and neuropharmacology of the spinal cord has provided alternative explanations. The present study was designed to examine the effects of spinally administered epinephrine on noxiously evoked activity of wide dynamic range neurons (WDR) in the dorsal horn of the spinal cord.

Extracellular single unit recordings were obtained from physiologically identified WDR neurons in decerebrate, spinal cord transected cats. A minimum of 4 hours transpired between the end of anesthesia used for initial surgical preparation and the beginning of drug studies. Physiologic parameters were monitored and maintained within normal limits. Spontaneous and stimulus evoked activity (radiant heat stimulus of 51^{0}C for 8 seconds applied to the peripheral receptive field on the hind paw foot pad) were studied during control and following the spinal administration of epinephrine. Epinephrine, 100 µg in .5 ml of physiologic saline, was placed on the spinal cord either following control studies or after naloxone reversal of suppression by spinally administered opioids.

The spinal administration of epinephrine following control studies produced a significant, long lasting suppression of noxiously evoked and spontaneous activity of WDR neurons. There was no obvious change in microscopically observed blood flow of the spinal cord during the period of suppression. The administration of epinephrine subsequent to naloxone reversal of spinal opioid suppression of WDR neurons produced a significant and rapid reduction in spontaneous and noxiously evoked activity of the WDR neurons studied. This suppression was also long lasting.

The co-administration of epinephrine with local anesthetics has been justified by its assumed effect on decreased vascular uptake of drugs. The present study indicates that spinally administered epinephrine has an effect upon both noxiously evoked and spontaneous activity of WDR neurons. Such an effect may be due to the interaction of epinephrine with descending adrenergic inhibitory systems. The results of the present study provide an alternative explanation for the enhancement of spinal block which is produced by the co-administration of epinephrine with local anesthetics.

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73.1 VASOPRESSIN: EFFECTS ON SYMPATHETIC PREGANGLIONIC NEURONES IN THORACIC INTERMEDIOLATERAL NUCLEUS OF THE CAT. J.L. Henry and S.B. Backman. Department of Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

Vasopressin has been implicated as a chemical mediator of direct pathways from the hypothalamus to lower structures regulating the cardiovascular system (Buijs, Cell Tiss. Res. 192:423, 1978), structures which may include the intermediolateral nucleus (ILN) of the spinal cord.

The present experiments were done to determine the effects of vasopressin on sympathetic preganglionic neurones (SPNs) in the ILN of spinal segments T_1-T_3 in cats anaesthetized with chloralose, paralyzed and ventilated artificially. Extracellular spikes were recorded from single units using the central barrel of multibarreled micropipettes. Other barrels contained glutamate (1M, pH 7.4), vasopressin (1mM in 165 mM NaCl at pH 5.5, Peninsula Laboratories) and at least one other neuroactive peptide (1mM in 165mM NaCl at pH 5.5, to control against changes in neuronal activity due to current or to pH). Pontamine Sky Blue was ejected into all positive sites of recording where it could then be identified in histological sections. All units included in this study were in the ILN, and all were identified as SPNs on the basis of their antidromic response to electrical stimulation of the sympathetic chain. Criteria for antidromic activation included a single all-or-none spike of invariant latency, the ability of this spike to follow high rates of stimulation and the cancellation of the evoked spike by a spontaneous (orthodromically propagated) spike.

Vasopressin, applied with positive currents of 50-125 nA to 22 SPNs, caused excitation in 14 units, had no effect on 3 and yielded inconclusive results with 5. Excitation typically consisted of a delayed (approximately 30 s), slow increase in ongoing rate of discharge. This response usually persisted for 1-5 minutes following termination of application. It was repeatable for any one neurone, yet was not mimicked by application of positive current through the barrel containing glutamate or through that containing another peptide.

In conclusion, these results raise the possibility that vasopressin is an excitatory mediator of synaptic transmission in the ILN, released perhaps from the terminals of fibres descending from supraspinal structures.

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73.2 DISSOCIATION OF CHANGES IN ARTERIAL PRESSURE AND PLASMA VASOPRESSIN BY ALTERING GABAERGIC FUNCTION IN THE REGION OF A1 NORADRENERGIC NEURONS. A.F. Sved, W.W. Blessing and D.J. Reis, Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Electrolytic and kainic acid lesions of the area of the ventrolateral medulla containing the A1 noradrenergic neurons elevate mean arterial pressure (MAP) and plasma vasopressin (VP) levels (Blessing et al., Science, in press). To determine whether a single population of cells in this area regulate both sympathetic vasomotor tone and VP release, we attempted to dissociate these responses using pharmacological probes. In the present study, agonists and antagonists of the inhibitory neurotransmitter GABA were injected into the A1 area and effects on MAP and VP measured. Rabbits were anesthetized with urethane (1.4 g/kg) and a femoral artery cannulated for monitoring MAP and sampling blood for VP. The dorsal surface of the medulla was exposed and microinjections (0.25 ul) of agents were made bilaterally through glass micropipettes into the A1 area. GABA injected into the caudal ventrolateral medulla produced a dose-dependent rise in MAP; this pressor response was localized to the region containing the A1 cells. Injections of the GABA agonist muscimol (10 nmol) into the A1 area rapidly elevated MAP (120 + 3 mm Hg before vs. 176 + 4 mm Hg 5 min after injection, n = 6; $p<0.01$). While these changes in MAP are similar to those produced by electrolytic lesions or kainic acid, muscimol did not elevate plasma VP (6 + 2 pg/ml before vs. 5 + 2 pg/ml 5 min after injection, n = 6). GABA (100 nmol) produced effects similar to muscimol. When bicuculline (0.1 nmol), a GABA antagonist, was injected into the A1 area there was an acute fall in MAP (49 + 11 mm Hg, n = 6, $p<0.01$), with MAP returning to baseline values approximately 5 min after injection. Changes in MAP were not due to changes in heart rate. After bicuculline injection, plasma VP levels were markedly elevated (8 + 1 pg/ml before vs. 106 + 13 pg/ml 5 min after injection, n = 5, $p<0.01$). The rise in plasma VP was not simply a consequence of the initial fall in MAP produced by bicuculline , since bicuculline elevated VP levels in rabbits with spinal cord transections (and MAP maintained with norepinephrine). We conclude: (a) vasodepressor cells in the A1 region are tonically inhibited by GABA; (b) GABA mechanisms in the A1 area which regulate the release of VP cannot be explained simply by a direct GABAergic inhibition of an A1 projection to the hypothalamus which inhibits VP release; and (c) the rise of MAP and plasma VP produced by lesions of the A1 area cannot be attributed to destruction of a single class of neurons in the A1 region.

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73.3 PRESSOR AND DRINKING RESPONSES TO ANGIOTENSIN II FOLLOWING SELECTIVE DEPLETIONS OF BRAIN CATECHOLAMINES IN DISCRETE RAT BRAIN NUCLEI. S. I. Bellin, R. K. Bhatnagar and A. K. Johnson. Depts. of Psychology & Pharmacology & The Cardiovascular Center, U. of Iowa, Iowa City, IA 52242.

A variety of electrophysiological, pharmacological, biochemical and lesion techniques have been used by many investigators in an attempt to demonstrate a causal role for brain catecholamines (CA) in the central control or regulation of body fluid homeostasis and cardiovascular hemodynamics. In the absence of any universal agreement regarding these cause and effect relationships, we have begun a systematic evaluation of changes in CA content limited to specific brain loci that may be correlated to specific alterations in behavioral (drinking) and pressor responses to angiotensin II (AII). In these studies, selective depletions of norepinephrine (NE), dopamine (DA) or both neurotransmitters, at the level of discrete brain nuclei, were obtained following intraventricular (IVT) injections of 6-hydroxydopamine in conjunction with pretreatment strategies employing pargyline and/or desmethylimipramine (Kostrzewa and Jacobowitz, 1974). Our pilot studies have confirmed that this protocol results in differential CA depletions in rat brain tissues at the regional level. Animals sustaining selective NE depletions limited to lateral and medial preoptic nuclei, the organum vasculosum of the lamina terminalis, the subfornical organ and the supraoptic and paraventricular nuclei (SON, PVN) no longer exhibited enhanced water intake to either systemic (3.0 mg/kg, S.C.) or IVT (100 ng/2λ) AII challenges. The magnitude of NE depletions in these animals ranged from 70-90% of values observed in controls. In addition, pressor responses to IVT AII (10, 50 or 100 ng/2λ) in these subjects were similarly abolished. Conversely, rats demonstrating selective DA depletions (deficits from 70-85% of control) in caudate, A14 and LPO nuclei only lost their ability to drink to systemic AII challenges. IVT AII administrations continued to elicit copious drinking and further resulted in an attenuation, but not an abolition, of the pressor response (13 mmHg rise vs a 25 mmHg elevation observed in non-depleted controls; $p \leq 0.01$). These data suggest that NE concentrations in brain nuclei at the level of the anteroventral third ventricle are intimately involved in the maintenance of body fluid homeostasis. It is also tempting to speculate that vasopressinergic, magnocellular neurons of the SON and PVN may contribute in a functional way to cardiovascular and body fluid integrity.

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73.4 CHANGES IN ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IN THE INTERMEDIATE LOBE OF THE PITUITARY GLAND AND IN SELECTIVE BRAIN STEM NUCLEI OF SPONTANEOUSLY HYPERTENSIVE RATS. J.M. Saavedra and C. Chevillard. Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Md. 20205

Angiotensin-converting enzyme (E.C. 3.4.15.1, KININASE II, ACE) activity was measured in young (4 weeks old) and adult (18 weeks old) spontaneously hypertensive rats (SHR) and in age-matched normotensive Wistar-Kyoto (WKY) control rats.

ACE activity was decreased in plasma and anterior lobe of the pituitary of SHR. Conversely, ACE activity was higher in the intermediate lobe of the pituitary gland of adult SHR, when compared to age-matched controls, and more so in young SHR. These changes did not appear to be due to a modified affinity of ACE for the substrate, Hippuryl-His-Leu. No change in ACE activity was observed in the posterior lobe of the pituitary gland (Table).

Lower ACE activities were found in SHR in selected areas of the brain stem, related to blood pressure regulation.

Young SHR showed decreased ACE activity in the A_1 area, and adult SHR presented decreased ACE activity in both the A_2 area and the anterior part of the nucleus tractus solitarius (Table).

No differences in ACE activities were noted in other brain stem areas, the locus coeruleus or hypothalamic nuclei.

	ACE activity pmol/ug protein/hr			*P < 0.05		
	4 weeks			18 weeks		
	WKY	SHR		WKY	SHR	
Pituitary						
Anterior lobe	172+14	98+9	*	72+2	44+2	*
Intermediate lobe	108+14	303+33	*	35+7	72+16	*
Posterior lobe	588+22	560+37		234+24	273+12	
Brain stem						
A_1 area	22+2	16+0.8	*	13+0.8	11+0.8	
A_2 area	29+4	28+3.0		17+1	12+1	*
Nucleus tractus solitarius	24+1	21+2		13+-0.3	11+0.8	*

Our results suggest that ACE might be involved in the mechanisms of the central regulation of blood pressure, and in the pathogenesis of genetic hypertension. Selective alterations of ACE activity in the intermediate lobe of the pituitary suggest that alterations in the metabolism of intermediate lobe peptides may also occur in SHR.

73.5 VASOPRESSIN AND CATECHOLAMINES ARE ELEVATED IN HINDBRAIN NUCLEI OF SABRA HYPERTENSION PRONE RATS. R. L. Zerbe*, G. Feuerstein, D. Ben-Ishay*, I. J. Kopin and D. M. Jacobowitz (SPON: L. Lemberger). Lab. of Clin. Sci., National Institute of Mental Health. Bethesda, MD. 20205.

Catecholamines (CA) and vasopressin (VP) have been detected in brain nuclei which are believed to be important in central regulation of blood pressure. Furthermore, some forms of genetic or experimentally induced hypertension are associated with changes in the content of these neurotransmitters in discrete brain regions. We have previously reported abnormalities in CA and VP content of forebrain nuclei of the Sabra strain of hypertension prone (SBH) and resistant (SBN) rats (Br. Res. Bull. 7: 671, 1981). To investigate catecholamines and vasopressin in baroregulatory areas in brains of the Sabra strain of hypertension prone (SBH) and resistant (SBN) rats, we measured norepinephrine (NE), epinephrine (E) and dopamine (DA) in the caudal (C) and rostral (R) nucleus tractus solitarii (NTS) and the locus caeruleus (LC), and measured VP in pooled NTS samples. Appropriate hindbrain regions from age matched 14-16 week old SBH, SBN and normotensive (SB) rats were microdisected, and the samples assayed for CA by radioenzymatic assay and for VP by radioimmunoassay. Blood pressure by tail plethysmography of SBH, SB and SBN rats was: 144 + 3, 130 + 3 and 112 + 3 mmHg respectively. NE and E content (pg/ µg protein + SEM) were greatest in SBH and lowest in SBN (*= p<0.05 vs SB):

	NE			E		
	CNTS	RNTS	LC	CNTS	RNTS	LC
SBN	11.3*	9.1*	10.2*	0.82*	0.66*	0.23*
	+0.6	+0.2	+0.6	+0.08	+0.03	+0.02
SB	5.0	3.8	4.7	0.30	0.34	0.10
	+0.2	+0.1	+0.3	+0.03	+0.02	+0.01
SBN	3.3*	2.9	2.9*	0.23	0.21*	0.05*
	+0.2	+0.2	+0.1	+0.02	+0.03	+0.01

DA was significantly higher in CNTS of SBH (0.29 + 0.04; SB= 0.15 + 0.03; SBN = 0.14 + 0.02 pg/µg prot.) but not in RNTS (SBH = 0.16 + 0.01; SB = 0.12 + 0.02; SBN = 0.12 + 0.02 pg/µg prot.) and LC (SBH= 0.52 + 0.07; SB= 0.51 + 0.06; SBN = 0.36 + 0.05 pg/µg prot.). VP was significantly higher in SBH (115.6 + 9.6 pg/mg prot.) and lower in SBN (29.6 + 3.9) than SB (38.3 + 2.7). We conclude that there are significant alterations in the content of CA and VP in the hindbrain nuclei of Sabra rats and that the changes in NE, E and VP parallel the divergent genetic sensitivity to hypertensive stimuli. Whether the observed differences in hindbrain content of these neurotransmitters are primary defects which contribute to the abnormal baroregulation of Sabra rats, or are a secondary response to these abnormalities, remains to be determined.

73.6 CENTRAL CATECHOLAMINERGIC ACTIVITY AFTER BILATERAL NTS FIBRE TRANSSECTION IN RATS. Z.Zukowska-Grojec*, D.C.Jimerson, M.A.Bayorh*, M.Palkovits*, I.J.Kopin. Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

Altered norepinephrine(NE) and epinephrine(EPI) contents in various brain regions has been found in some animal models of hypertension. However, the role of these changes in the development of hypertension remains unclear. To investigate further this phenomenon we studied the effect of acute hypertension produced by bilateral NTS fibre transsection on the levels of NE,EPI and their metabolites: 3, 4-dihydroxyphenylglycol(DHPG)and 3-methoxy-4-hydroxyphenylglycol (MHPG) in NTS,A2,A1 area and periventricular nucleus(NPV). 29 tail-artery cannulated rats(250g) were divided into 4 groups: NTS transsected,saline-pretreated (N-S); NTS transsected, chlorisondamine(CHLOR,10mg/ kg,s.c.)-pretreated(N-C); sham, saline-pretreated (S-S) and sham,CHLOR-pretreated(S-C). One hr after CHLOR mean blood pressure(MBP)and heart rate(HR) decreased significantly(p<.001 as compared to basal) NTS transsection produced acute increase in MBP and HR in both N-S(68±3 mm Hg and 97±19 beats/min) and N-C(61±11 mm Hg and 108±30 beats/min) which lasted for 15 min at which time rats were killed. Brain areas were punched out by method of Palkovits and in each area NE and EPI (radioenzymatic assay)and total MHPG and DHPG(gas-chromatography mass-spectroscopy)were measured and values expressed in ng/mg protein. No significant changes in metabolites were found in any of areas studied. NTS section decreased NE content of the NTS from 19.9±3.6 to 11.5±2.0 in saline-treated rats and from 21.3±1.9 to 8.1±1.0 ng/mg protein in CHLOR-treated rats. In the A2 area, CHLOR treatment elevated NE content from 12.0±0.9 in saline-treated animals to 27.3±4.7ng/mg. In NTS sectioned rats the levels of NE were intermediate:18.4±3.4 in saline-treated, 15.4±1.2 in CHLOR treated rats, respectively. These results can be explained by the presence in the A2 area of two groups of NE-containing structures. In one group NTS fibre section appears to have effects similar to that in the NTS, increasing adrenergic activity and lowering NE levels. The other group appears to be inhibited by CHLOR treatment, resulting in increased levels of NE. Combined CHLOR treatment and NTS section has effects similar to that of NTS section alone, which increase NE in one group of A2 NE-containing structures and lowers it in the other resulting in a smaller net increase in NE than with CHLOR treatment alone. The changes in NE in the NPV and the differences among levels in NE metabolites in the areas examined were not significantly different. This might be due to the small areas and low metabolites levels. This results in high variation which could obscure the real changes in the small amounts of metabolites measured. Furthermore, since glycols diffuse freely in brain tissue, localized changes might be obscured by diffusion into and from adjacent areas.

73.7 EFFECT OF SPECIFIC LESION OF SEROTONERGIC NEURONS IN THE DORSAL RAPHE NUCLEUS AND MEDIAN RAPHE NUCLEUS ON THE PRESSOR RESPONSE TO ELECTRICAL STIMULATION. S. E. Robinson, F. Austin*, and D. Shamel*. Department of Pharmacology, Medical College of Virginia, Richmond, VA 23298.

Electrical stimulation of the dorsal raphe nucleus (DRN) and of the median raphe nucleus (MRN) increases blood pressure in the rat (Smits, J.F.M. et al, Life Sciences, 23:173, 1978; Kuhn, D.M. et al, J. Pharmacol. Exp. Ther., 214:403, 1980). In order to determine if the pressor response is due to the activation of serotonergic neurons, the effect of specific lesion of serotonergic neurons in either the DRN or the MRN with 5,7-dihydroxytryptamine (DHT) was studied on the cardiovascular response to electrical stimulation of these areas. DHT (10 µg in 2 µl) was injected into the brains of desipramine-pretreated rats (25 mg/kg, i.p.) at the DRN (coordinates: AP +0.2, L 0.0, V -0.8) or the MRN (coordinates: AP +0.2, L 0.0, V -2.8). Control animals were injected at the same site with the vehicle (0.2% ascorbic acid, 0.9% saline). Eleven days later, the area receiving the local injection was stimulated electrically in urethane-anesthetized rats with a concentric bipolar electrode (stimulation parameters: 50 Hz, 0.3 msec pulse duration, 5 sec train duration, 50-150 µA) and blood pressure was recorded by use of a cannula inserted in the femoral artery. The extent of lesion was determined by measuring the level of serotonin (5HT) in brain areas. There was a significant reduction in the pressor response to raphe stimulation in rats injected with DHT in the DRN (40% to 60% reduction as compared to vehicle-treated controls). 5HT was reduced in the cortex (26% of control), striatum (45% of control), but not in the spinal cord (97% of control). There was no difference in the pressor response to i.v. phenylephrine (50 µg/kg). However, preliminary data suggest that specific lesion of 5HT neurons in the MRN does not prevent the pressor response to MRN stimulation and may even increase the pressor response to stimulation of this area and to i.v. phenylephrine.

Thus, it appears that at least part of the pressor response to electrical stimulation of the DRN is due to stimulation of 5HT neurons. However, the situation in the MRN is not as clear. Either 5HT neurons are not involved in the pressor response to MRN stimulation or there is a supersensitive response to the stimulation of the remaining 5HT neurons. This increased response may be at least partially due to an increased response to stimulation of peripheral adrenergic receptors. (Supported by a Grant-in-Aid from the American Heart Association with funds contributed in part by the American Heart Association, Texas Affiliate, Inc.).

73.8 Serotonergic Neurons in the dorsal raphe stimulate renin secretion from the kidney via a factor in the blood.

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In previous studies (Van de Kar, et al., JPET 219:85-95, 1981, Brain Res. 235:223-243, 1982, Karteszi, et al., Neuroendocrinology 34:1982) we provided evidence that serotonergic neurons that originate in the dorsal raphe nucleus and terminate in the mediobasal hypothalamus stimulate renin secretion. The serotonin mediated increase in plasma renin activity (PRA) however was not prevented by hypophysectomy. We have also demonstrated that adrenalectomy, adrenal ennucleation, renal denervation, spinal transaction at the T_2 level or pretreatment with the sympathetic blocker bretylium did not prevent the increase in PRA after injection of the serotonin releaser p-chloroamphetamine (PCA) (Van de Kar, et al., Soc. Neurosci., 7, 45:6, 1982) PCA induced increase in PRA was also not prevented by pretreatment with the muscarinic receptor blocker methylatropine. The present studies were designed to verify that the increase in PRA is due to increased renin secretion from the kidney and to find how the stimulus to increase renin secretion is transmitted from the brain to the kidney. Rats were nephrectomized bilaterally and, 20 hr later received injections of either PCA (10 mg/kg i.p.) or saline. Nephrectomy completely prevented the effect of PCA on PRA suggesting that the kidney is the only source of renin that responds to the serotonergic stimulus. In order to test whether a blood borne factor mediates the effect, a transfusion experiment was performed. The donor rats were nephrectomized and received either saline or PCA (12 mg/kg i.p.) injections. Plasma from the donor rats was then administered at a dose of 12 ml/kg i.p. at various times before sacrifice to recipient rats. Administration of the plasma from PCA treated donor rats caused a significant increase in PRA which peaked at 30 min and disappeared at 60 min after administration. This PRA time response is different from the response to i.p. injection of PCA which is delayed and does not increase until 60 min after administration. However, to verify that the effect was not due to residual PCA in the donor plasma causing serotonin release, a group of acceptor rats were pretreated with the serotonin depleting drug PCPA (300 mg/kg i.p., 72 hr). Again injection of plasma from PCA treated donor rats caused a significant increase in PRA. These results provide evidence that brain serotonin stimulates renin secretion from the kidney via a factor which is transported in the blood.

73.9 EFFECTS OF CENTRALLY ADMINISTERED PROSTACYCLIN AND 6-KETO PROSTAGLANDIN $F_{1\alpha}$ ON MEAN ARTERIAL PRESSURE OF RATS. Nancy J. Kenney, Dept. of Psychology, Univ. of Washington, Seattle, WA 98195.

Prostacyclin (PGI_2) delivered into the lateral ventricles of the brains of unanesthetized, unrestrained rats at a dose of 1 µg/0.5 µl with injections repeated each min for 5 min caused a marked reduction of mean arterial pressure (MAP). MAP was reduced significantly within 1 min of the onset of PGI_2 treatment ($F(1,5)=6.35$, $p<.05$ compared to MAP changes following injection of the PG carrier solution) and reached a maximum decrease of 15.6 ± 3.8% from preinjection baseline at the end of the 5-min PGI_2 injection period. MAP returned to levels observed following control injections within 2 min after the end of PGI_2 treatment.

Such PGI_2 treatment completely reversed the increase of MAP which resulted from central injection of 5 ng angiotensin II. Change of MAP from baseline of angiotensin-treated rats was significantly attenuated within 1 min of the onset of PGI_2 treatment ($F(1,5)=18.70$, $p<.01$). Seven min after the onset of PGI_2 treatment, changes of MAP of rats when treated with angiotensin plus PGI_2 had returned to the levels observed following the injection of angiotensin plus PG vehicle.

Pretreatment with probenecid blocks fatty-acid transport across biological membranes and, thus, should prevent leakage of centrally injected PGI_2 to the periphery. Such pretreatment resulted in a significant augmentation of both the degree ($F(1,8)=6.08$, $p<.05$) and the duration ($F(9,72)=13.79$, $p<.005$) of the central PGI_2 induced depressor effect, indicating that centrally injected PGI_2 can reduce MAP through direct action within the brain rather than requiring leakage from the brain to the periphery.

Intracerebroventricular (IVT) injection of 6-keto-prostaglandin $F_{1\alpha}$ (6-K-$PGF_{1\alpha}$, 1 µg/0.5 µl repeated each min for 5 min), a major stable degradation product of the unstable PGI_2, resulted in a significant increase of MAP above that observed following control injections of the carrier solution. MAP was significantly elevated above control levels 7 min after the onset of IVT 6-K-$PGF_{1\alpha}$ injections ($F(1,7)=7.06$, $p<.05$) and remained significantly elevated for approximately 10 min.

Thus, PGI_2 may act within the brain, without leakage to the periphery, to reduce MAP and to counteract the effects of centrally acting hypertensive agents such as angiotensin II.

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73.10 GABA INHIBITION OF CENTRAL AII-INDUCED AVP-DEPENDENT PRESSOR MECHANISMS. Tim Brennan* and J.R. Haywood* (SPON: W.B. Stavinoha). Dept. Pharmacology, UTHSC, San Antonio, TX 78284.

We have previously demonstrated that intraventricular (IVT) gamma-aminobutyric acid (GABA) inhibits central angiotensin II (AII) pressor responses. This inhibition by GABA is dose-dependent and rapidly reversible. The present studies were undertaken to determine the mechanism of this inhibition by GABA.

In conscious rats, IVT administration of 50, 150, and 500 ng of AII increases mean arterial pressure (MAP) 11.4±1.7, 15.8±1.3 and 19.1±2.7 mmHg, respectively. After autonomic blockade with chlorasondamine, the pressor responses of IVT AII were augmented so that 50, 150, and 500 ng of AII increased MAP 19.5±1.2, 25.3±2.6, and 33.0±2.0 mmHg, respectively. After ganglionic blockade, pretreatment with 25 µg of GABA does not change baseline MAP, but reduces the pressor response of IVT AII to 9.3±2.7, 17.7±4.7, and 21.8±3.2 mmHg, respectively. Administration of 100 µg of GABA results in a further shift to the right of the AII dose-response curve. In addition, following chlorasondamine treatment, the pressor response of 500 ng of AII (IVT) is reduced from 33.2±2.0 to 6.5±1.5 mmHg by $d(CH_2)_5$VDAVP, a specific vascular antagonist of arginine-vasopressin (AVP).

In another series of experiments, rats were treated with $d(CH_2)_5$VDAVP only. The pressor responses of 50, 150, and 500 ng of AII after vascular AVP blockade were reduced approximately 60% from control to 4.8±0.6, 7.2±2.0, and 7.6±1.3 mmHg, respectively. Following pretreatment with 100 µg of GABA (IVT), these pressor responses of IVT AII were not changed.

We conclude that IVT AII increases arterial pressure through both AVP and the sympathetic nervous system, as previously reported. After ganglionic blockade, the central AII pressor response is mediated through AVP, and this AII-induced AVP-dependent pressor response is sensitive to low doses of IVT GABA. After AVP blockade, the neurogenically-mediated central AII pressor response is not apparently sensitive to GABA. (Supported by American Heart Association, Texas Affiliate and NIH HL 26993).

73.11 EVIDENCE THAT A GABAERGIC MECHANISM AT THE VENTRAL SURFACE OF THE MEDULLA INFLUENCES BLOOD PRESSURE AND HEART RATE IN THE RAT. J.R. Keeler* and C.J. Helke. (SPON: J. M. Sarvey). Dept of Pharmacol., Uniformed Services Univ. of the Health Sci., Bethesda, MD 20814.

The ventral surface of the medulla oblongata (VSMO) has been implicated in the central regulation of the cardiovascular system. Electrical stimulation of the area raises mean arterial blood pressure (MAP) and bilateral destruction lowers MAP. Three specific areas of the VSMO (rostral, intermediate, and caudal) are defined according to the cardiovascular and respiratory effects elicited by topical application of various agents. GABA and the GABA receptor agonist, muscimol, decrease MAP and heart rate (HR) when applied to the intermediate area. The intermediate area, located lateral to the pyramids and caudal to the trapezoid bodies may be a target site for drug action. Yamada et al (Fed Proc 41:1764, 1981) report that the cardio-respiratory depression induced by intravenous pentobarbital is via a GABAergic mechanism at this locus.

Whereas the above mentioned studies were performed in the cat, many of the corresponding neuroanatomical and neurochemical studies were done in the rat. Therefore, the present studies were designed to evaluate GABAergic systems at the VSMO in the rat and their influence on MAP and HR. Specifically, we determined the sensitivity of the VSMO in the rat to GABA and muscimol, localized the specific site of action and assessed the role of GABA at the VSMO in normal cardiovascular function. Chloralose-urethane anesthetized rats were artificially respired, and the VSMO (from the bulbopontine junction to C1) was carefully exposed with the aid of a stereomicroscope. MAP, HR, ECG, rectal temperature, and arterial blood gases were monitored and recorded. Localization of drug application was verified at the end of each experiment with dye application. GABA (0.234 µmol in 10 µl) injected over the entire VSMO produced a fall in MAP of 39 ± 6 mmHG and HR of 26 ± 7 beats/min. GABA-soaked pledgets (0.5 x 0.5 mm) were placed lateral to the pyramids in 7 positions rostro-caudally and bilaterally. The cardiovascular responses showed that the area of greatest sensitivity is just caudal to the trapezoid bodies. Both GABA (0.0234-2.34 µmol) and muscimol (0.044 - 1.31 nmol) applied to this intermediate area produced dose-related decreases in MAP and HR. The effects of GABA and muscimol were reversed by application of the GABA receptor antagonist, bicuculline methiodide (0.59 nmol). Bicuculline alone produced an increase in MAP (36 ± 10 mmHg) and HR (85 ± 10 beats/min) when applied to this site on the VSMO.

These data suggest that a GABAergic mechanism at the intermediate area of the VSMO exerts a tonic influence on autonomic activity to the cardiovascular system. Furthermore, these results show that the rat is a suitable model to study the VSMO and its role in cardiovascular function. Supported by USPHS Grant #HL-26849.

73.12 ANTAGONISM OF GLUTAMIC ACID DIETHYLESTER TO THE EXCITATION OF NUCLEUS TRACTUS SOLITARII NEURONS BY L-GLUTAMIC ACID OR BY SYNAPTICALLY STIMULATING VAGAL AFFERENT FIBERS. A.R. Granata and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

We have proposed that L-Glutamate (L-Glu) is released by neurons in the nucleus tractus solitarii (NTS) which mediate reflexes from baro- and possibly other cardiovascular mechanoreceptors (Reis et al., J. Auto. Nerv. Sys., 3:34, 1981). To test this hypothesis further, we sought a) to determine whether an L-Glu antagonist, glutamic acid diethylester (GDEE) would block the excitation of "cardiovascular" neurons in the NTS by vagal stimulation or by acute increase in systemic blood pressure; and b) to prove that GDEE is a competitive glutamatergic antagonist in neurons in NTS.

Rats were anesthetized with urethane (1.5 g/kg i.p.), paralyzed and ventilated. The cervical vagus, transected with the central end placed on a stimulating electrode, was electrically stimulated with pulses of 0.1 msec duration, 5 Hz and 120 µA. An extracellular recording electrode protruding 5-15 µm beyond a multibarrel pipette was inserted into the NTS. Recording sites were marked by passing fast green from the recording barrel. Vagal stimulation modified the firing rate (FR) of 60/75 (80%) spontaneously active units. Vagal stimulation increased the FR in 33 and decreased the FR in 27 neurons. The ten neurons which increased their FR to vagal stimulation were considered as "cardiovascular" since they also increased their FR when arterial pressure was elevated by l-NE. Nine out of the ten cardiovascular neurons were histologically localized to NTS. In these neurons the latency of the first spike evoked by vagal stimulation varied between 3-6 msec (Avg: 4.2 msec); the number of spikes in each burst (usually 1-3) increased with increasing stimulus intensity; with repeated stimuli, the latency was unstable and spikes did not follow stimulation frequencies >100 Hz. In all 10 cells systemic GDEE (180 mg/kg i.v.) reversibly blocked both the vagally evoked spikes and the reflex bradycardia and hypotension. In contrast, GDEE did not block the hypotension and bradycardia elicited by electrical stimulation of the trigeminal complex.

In another experimental group, we found that in a total of 23 neurons l-Glu released with a micropump from a multibarrel pipette, increased the FR. In 18/23 cells, GDEE released 15 sec before l-Glu reversibly blocked the excitatory effect of this agonist.

We conclude that the presumably monosynaptic excitation of some NTS neurons by cardiopulmonary vagal afferents is blocked by an antagonist of l-Glu. The results are consistent with the hypothesis that l-Glu is a transmitter released in NTS in response to baroreceptor stimulation.

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A.R. Granata is a Fellow from Consejo Nacional de Investigaciones Cientificas y Tecnicas de La Republica Argentina.

74.1 SYMPATHETIC PREGANGLIONIC NEURONS AND VISCERAL PRIMARY AFFERENTS SUPPLYING THE PELVIC ORGANS IN THE CAT. J.J. Oravitz*, C. Morgan, I. NADELHAFT, and W.C. DE GROAT. (Spon: S.J. Yao) Depts. Neuro. Surg. & Pharm., Univ. Pitt. Sch. Med. & V.A. Hosp., Pitt., PA

In recent papers we have described the distribution of sacral parasympathetic preganglionic neurons (PGN) and primary afferents innervating the pelvic viscera in the cat. These organs also receive a sympathetic input via the hypogastric nerve (HGN), lumbar colonic nerve (LCN) and the lower sympathetic chain (SC). The present research examines the central distribution of sympathetic PGN and visceral afferents carried by these nerves.

HRP was applied to the cut HGN and LCN and injected into the sacral SC ganglia. HRP was identified in dorsal root ganglia (DRG) from all three nerves, but only in HGN and LCN expts was reaction product intense enough to trace within the spinal cord. In HGN expts DRG cell counts, density of central afferent axons and PGN counts, were highest in L2-L4, although afferents were detected from T13-L6. DRG counts and central afferents in LCN expts were heaviest in L3-L4. In contrast, DRG cells in the SC expts were broadly distributed (L1-S3) and were not closely correlated with the distribution of PGN. Central afferent projections from the HGN and LCN were similar. HRP was seen in Lissauer's tract (LT), in superficial lamina I, lateral and medial lamina V, medial lamina VII, and the dorsal gray commissure. PGN projecting in HGN (39O) or SC (35OO) showed differences in location and morphology while no PGN were labeled via the LCN. The majority of cells supplying the HGN were transversely oriented, either elongated or round in shape and located in clusters in the principal intermediolateral nucleus (IMLp). Smaller numbers were found in the ventral horn, the nucleus intercalatus (IC) and the IC disseminata (ICd). Very few rostrocaudal oriented cells, funicular cells, or IC ependymal cells (ICe) were identified. On the other hand, in the SC expts there were many rostrocaudal, funicular, and ICe cells; and while the majority of these were found in the IMLp, they tended to be located more laterally than HGN neurons

In summary, central projections of HGN and LCN afferents are similar to those of pelvic nerve afferents in the sacral cord. These nerves all send their axons into LT, collaterals of which form a shell around the dorsal horn and enter the autonomic nuclei. LT thereby serves as a major visceral afferent pathway integrating autonomic reflexes over a considerable length of spinal cord from T13-L7 for the sympathetic nerves and L4-Cx7 for the parasympathetic.

The efferent components of the HGN, LCN and SC are not similar. The LCN contains no PGN axons while the HGN and SC exhibit differences in both the location and morphology of their neurons which may reflect differences in function.

74.2 PROJECTIONS FROM THE FOREBRAIN TO CARDIOVASCULAR AREAS IN THE NUCLEUS OF THE SOLITARY TRACT IN THE CAT. M. B. Gutman*, J. Ciriello, M. M. Caverson* and F. R. Calaresu (SPON: P. E. Cooper). Department of Physiology, University of Western Ontario, London, Canada N6A 5C1.

It is well established that specific areas in the nucleus of the solitary tract (NTS) are primary sites of termination of afferent fibers of the buffer nerves and that these areas are important in the mediation of cardiovascular responses associated with the activation of baroreceptor and chemoreceptor reflexes. It has also been shown that activation of forebrain areas alter these cardiovascular reflex responses, possibly through influencing neuronal activity in the NTS, although the locations of these forebrain areas have not been identified unequivocally. In the present study experiments were done in cats to determine by the horseradish peroxidase (HRP) method, the location of neurons in the forebrain which project to the NTS. HRP was allowed to diffuse from glass micropipettes (50-150 µm, internal diameter) into regions of the NTS previously identified to receive primary projections from buffer nerves (JANS, 4; 43-61, 1981). After a survival period of 70-120 h, forebrain sections were cut at 40 µm and processed according to the tetramethyl benzidine method. Labelled cells were observed primarily throughout the rostrocaudal extent of the hypothalamus, bilaterally: in the region of the anterior hypothalamic area, the periventricular area, the dorsal hypothalamic area, the ventromedial hypothalamic area, the posterior hypothalamic area and the fields of Forel. The paraventricular nucleus was observed to contain the majority of labelled cells, predominantly ipsilaterally. Additional labelled cells were identified in the central nucleus of the amygdala, bilaterally, with an ipsilateral predominance. An occasional labelled neuron was also observed in the rostral region of the contralateral central grey. These forebrain sites identified to project to known areas of integration of cardiovascular reflexes in the NTS are likely candidates for a functional role in the supramedullary neural control of the circulation.

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74.3 PROJECTIONS FROM THE VENTROLATERAL MEDULLA TO THE DORSOLATERAL PONS AND PERIAQUEDUCTAL GRAY IN THE RAT. S. McKellar* and A.D. Loewy. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Projections from the region of the A1 catecholamine cell group were studied by the autoradiographic method. The cells of origin were identified by the retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA/HRP). Taken together, these methods showed that cells in or near the nucleus ambiguus project bilaterally to the Kölliker-Fuse nucleus and, more weakly, to lateral portions of the parabrachial area. A mainly ipsilateral projection to the locus coeruleus arises mainly from a more medial area, near the lateral tip of the inferior olive at the level of the retrofacial nucleus. Other fibers project to lateral and ventrolateral portions of the periaqueductal gray, especially contralaterally. The cells of origin are similar in distribution and appearance to cells of the A1 catecholamine cell group as seen by histofluorescence. The motor trigeminal nucleus receives fibers from the reticular formation dorsomedial to the nucleus ambiguus.

In further experiments, WGA/HRP was combined with catecholamine histofluorescence to demonstrate the retrograde marker in the same section with fluorescence. This technique clearly shows a projection to the bed nucleus of the stria terminalis from A1 cells. With rare exceptions, however, the A1 cells were not labeled by injections of WGA/HRP anywhere in the dorsolateral pons or even in the periaqueductal gray. We conclude that the projections described above arise mainly or exclusively from non-noradrenergic cells, while the A1 group itself projects to more rostral targets in the basal forebrain.

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74.4 An efferent projection from the Area Postrema and the caudal medial Nucleus of the Solitary Tract to the Parabrachial Nucleus in rat. R. E. Shapiro* and R. R. Miselis Dept. Anatomy, School of Vet. Med., Univ. Pennsylvania, Philadelphia, Pa., 19104

The Area Postrema (AP) and the adjacent caudal medial subnuclei of the Nucleus of the Solitary Tract (cmNTS) constitute a major terminal zone for sensory fibers of the vagus nerve. This fact, when combined with the lack of a blood-brain barrier within AP, provides anatomical support for this region as a visceral sensor both neurally and humorally. The transduction of this sensory information may depend upon neural projections from the AP/cmNTS. We find an efferent pathway from the AP/cmNTS to a set of discrete subnuclei within the pontine parabrachial complex (PB). Adult male Sprague-Dawley rats were injected with HRP conjugate tracers via micropipette (5u tip) under hydraulic pressure or by iontophoresis. HRP (Sigma VI) was conjugated to wheat germ agglutinin (E-Y) or choleragen (List) according to the method of Avrameas & Ternyck (1971). Volumes of 30 to 40 nl of either tracer were applied in concentrations of approx. 1% to 5% to the PB region or the AP. Animals were sacrificed 24 hrs. post-injection and the brains processed according to the TMB protocol of Mesulam. Injections confined to AP/cmNTS produced TMB reactive fibers traceable ventrolaterally through the reticular formation. These fibers then course rostrally with the rubrospinal tract until they enter the rostral pons where they turn dorsally within the lateral lemniscus to reach the lateral division of the PB towards the middle of its rostro-caudal extent. When viewed in transverse sections, two terminal fields within the rostral half of the lateral PB become apparent. The entering fibers first form a large dense oval-shaped field visible immediately dorso-lateral to the lateral half of the brachium conjunctivum. Then, continuing rostrally, the TMB reaction product becomes concentrated more dorsomedially in a second, smaller (approx. 0.1 mm across), dense patch directly ventral to the overlying spinocerebellar tract. This rostral field coincides with a cluster of relatively large neurons (approx. 16 u in width) readily distinguishable in nissl stained material. When the rostral half of the lateral PB is injected with HRP conjugate, retrogradely filled neurons can be detected in the AP and in the adjacent subnuclei of the NTS. The PB complex has extensive efferent projections to limbic structures and other regions presumed to be involved in autonomic function (Saper and Loewy, 1980). The AP/cmNTS projection to PB could provide an avenue for the modulation of central autonomic systems by visceral sensory information. [Supported by GM 27739 and USPHS 5T32 GM07517]

74.5 BRAIN STEM LESIONS MODULATE THE FASTIGIAL NUCLEUS PRESSOR RESPONSE IN ANESTHETIZED BEAGLES. K.J. Dormer, R.D. Foreman, J.A. Andrezik and R.J. Person. Depts. of Physiology and Biophysics and Anatomical Sciences, University of Oklahoma, Oklahoma City, OK 73190.

In order to identify the brain stem site(s) which mediate the fastigial nucleus (FN) hypertensive-tachycardia response (FPR), regions previously identified by autoradiography as receiving direct fastigiobulbar projections (Andrezik et al., Fed. Proc. 41:1517, 1982) and other areas were lesioned; the effects on the FPR were recorded. Beagles (n=10) were anesthetized with aqueous alpha-chloralose (110 mg/kg) and artifically ventilated. A femoral cannula was used to obtain arterial pressure and a solid-state, catheter-tip transducer was retrogradely placed into the left ventricle for determination of left ventricular pressure (LVP) and maximal dLVP/dt (contractility index). The electrocardiogram and heart rate (HR) were also recorded. The rostromedial FN was stereotaxically implanted with concentric semimicroelectrodes and the FPR was produced by stimulating with square wave pulses (150 μs, 80 Hz, 200-500 μA. Next, radiofrequency (RF) lesions (75°C/60 sec) were bilaterally placed in one of the following areas: brachium conjunctivum, dorsolateral, or medial pontine reticular formation, and the following nuclei: gigantocellularis, paramedian reticular (PRN), lateral reticular (LRN), tractus solitarius (NTS), and area A-5 lateral to the facial nucleus. The FN was stimulated just prior to and 10-15 minutes following the lesion placement, first contralateral then ipsilateral to the FN stimulation. Abolition of the FPR was only observed following bilateral lesions in the A-5 area or brachium conjunctivum. Apparently the A-5 region on each side is sufficient for transmission of the FPR because unilateral lesions of area A-5 only partially reduce the FPR. No changes in the FPR were observed due to lesions in PRN or LRN, however, lesions of NTS 3 mm rostral to obex augmented the FPR with sustained tachycardia, greater rate of pressure development and increased contractility. Elevations in resting HR, AP, LVP and dLVP/dt were observed with lesions in NTS and an area 3 mm dorsal to A5. Based on autoradiography and our lesion studies, we conclude that the pathway from the FN to the A-5 region is bilateral and disynaptic or polysynaptic. The A-5 region and its descending sympathetic connections makes a major contribution to the FPR. Supported by HL 24082.

74.6 RELATIONSHIPS BETWEEN CARDIAC VAGAL AND SYMPATHETIC DISCHARGES: RHYTHMIC FLUCTUATION IN RELATION TO RESPIRATION AND HEART BEAT. N. Terui*, M. Kollai and K. Koizumi. Dept. of Physiol., State University of New York, Downstate Medical Center, Brooklyn, N.Y. 11203.

Although fluctuations in the autonomic nerve activity relative to respiratory and cardiac rhythms are well known, the relationship between such rhythmic activity in the two autonomic outflows to the heart has not been well studied. We investigated this problem by recording simultaneously from both cardiac vagal and sympathetic nerves in chloralose anesthetized and artificially ventilated dogs. Under normal conditions fluctuations in activity relative to the respiratory rhythm was present in all cardiac sympathetic and vagal nerves. As shown previously[1] the sympathetic activity increased during phrenic discharges, while vagal discharges increased in the absence of phrenic or during expiratory muscle nerve activity. Although the peak of vagal excitation occurred at various times between successive phrenic bursts, the relationship between the two nerve activity was always clearly reciprocal and remained so within wide range of phrenic cycle (8-50/min). Fluctuations in the autonomic nerve activity relative to cardiac rhythm were also found in practically all cases. The timing of the rhythmic activity of cardiac sympathetic and vagal efferents with respect to the arterial pulse or R wave of EKG was not exactly reciprocal; under normal conditions the peak of vagal excitation occurred earlier than onset of the inhibition of sympathetic activity so that during a part of diastoles the activity of both nerves was much reduced or ceased simultaneously. This relationship remained the same at varied heart rate (63-157 beats/min). After baroreceptor denervation cardiac rhythm-related fluctuations disappeared completely as expected, while those related to the phrenic activity were unaltered. Disappearance of rhythmic fluctuations caused by one factor (respiratory or cardiac rhythm) in a certain condition did not alter the rhythmic activity caused by another factor. Likewise, disappearance of rhythmic activity patterns in either the vagus or sympathetic nerve did not affect rhythmic fluctuation seen in another nerve. In addition, it was found that changes in relationships between the discharge patterns of the two nerves with respect to respiration and the heart beat occurred under subnormal conditions and that these played an important role in affecting cardiac functions. (Supported by USPHS Grant, NS-00847)

[1] J. auton. Nerv. Syst., 1:33-52,'79; 4:135-148,'81.

74.7 INTERACTIONS BETWEEN BARORECEPTOR AND SYMPATHETIC PATHWAYS. Robert B. McCall and Stephen J. Humphrey. Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The interactions between baroreceptor and sympathetic pathways were determined in the anesthetized cat using computer summation techniques. Single pulse electrical stimuli applied to baroreceptor afferent nerves produced an early and late period of computer-summed positivity (i.e. inhibition) of sympathetic nervous discharge (SND) recorded from the external carotid or inferior cardiac nerves. The temporal characteristics of the positivities evoked by stimulation of either vagal or aortic depressor afferents were identical. In addition, the time course of inhibition of SND recorded from either sympathetic nerve appeared similar. The shape of the late inhibition closely resembled that of the 3 cycle/s cardiac locked slow wave of SND, suggesting that the late inhibition resulted from the termination of a complete slow wave of SND. Similar observations have led others to suggest that the late period of inhibition reflects the ability of baroreceptor afferents to entrain SND to the cardiac cycle.

Several observations in the present study, however, indicate that the slow wave of SND can be disassociated from the late inhibition. First, the late period of baroreceptor-induced inhibition summated with the cardiac locked slow wave of SND during all phases of the cardiac cycle. Second, increases in the intensity of baroreceptor stimulation resulted in an increase in the amplitude and duration of the late inhibition. Third, alterations in the periodicity of SND failed to effect the late phase of inhibition. Thus, the late inhibition was not altered when periodicity of SND spontaneously shifted from a 3 cycle/s to a 10 cycle/s rhythm. Finally, intravenous picrotoxin blocked the late period of baroreceptor induced inhibition, but enhanced the cardiac locking of SND. Administration of picrotoxin into the fourth ventricle, but not intrathecally, produced similar effects and were seen in the absence of blood pressure changes. These data indicate that the late period of inhibition reflects the ability of baroreceptor afferent nerves to modulate the amplitude of central sympathetic outflow, regardless of its periodicity. In addition, the central baroreceptor pathways involved in amplitude modulation and entrainment of SND to the cardiac cycle are distinct and are located at the level of the brain stem. Finally, the baroreceptor pathway involved in amplitude modulation of SND appears to be mediated at least in part by GABA.

74.8 HYPOTHALAMIC KNIFE CUTS ATTENUATE THE NATRIURESIS INDUCED BY WATER DEPRIVATION. Steven L. Bealer, Joan T. Crofton*, Leonard Share*. Dept. Physiology & Biophysics, University of Tennessee Center for the Health Sciences, Memphis, TN 38163

Electrolytic ablation of the periventricular tissue surrounding the anteroventral third ventricle (AV3V) results in acute alterations in sodium balance, chronic hypernatremia, expanded extracellular fluid volume, and attenuated natriuretic responses to a variety of experimental manipulations. The present experiments investigated the effects of severing the medial neural connections coursing from the AV3V region on the natriuretic response induced by water deprivation. A retractible wire knife was used to make knife cuts in the coronal plane anterior to the paraventricular nuclei, extending to the floor of the third ventricle. Control rats underwent similar surgical procedures, but did not receive cuts. Five days following surgery, animals were housed in metabolism cages and allowed one day ad libitum access to food and water. Animals from both groups were then deprived of water for 48 hrs. Daily food ingestion, urine volume, sodium excretion, potassium excretion, and urine osmolality were measured. Animals with knife cuts and control rats which were water deprived had similar decreases in food ingestion and urine volume, as well as equivalent increases in urine osmolality. The percent of dietary sodium present in the urine was not different between groups (80 ± 5% for controls; 86 ± 4% for cuts) during ad libitum access to food and water. However, water deprivation caused a significantly greater natriuresis in control rats (154 ± 6%) than in animals with knife cuts (118 ± 9%) during the first day of water deprivation. Urinary sodium excretion was similar between groups during the second day of deprivation (87 ± 9% of dietary intake for controls; 77 ± 8% for cuts). During the two days of deprivation the mean total sodium loss relative to ingestion by control animals was 359 ± 89 μEq/100 g BW, and 26 ± 112 μEq/100 g BW for cut animals. However, animals with knife cuts excreted significantly more potasium than by control operated rats during deprivation. Plasma sodium concentration in animals with knife cuts was significantly greater following water deprivation (164 ± 19 mEq/l) than similarly deprived control rats (145.5 ± 1.0 mEq/l). These data show that the natriuresis induced by water deprivation is attenuated by coronal knife cuts posterior to the organum vasculosum lamina terminalis, and indicate that this brain region contains a neural pathway critical for normal sodium regulation. (This research supported in part by USPHS grants HL 25877, HL 12990 and from the American Heart Association, Tennessee Affiliate)

74.9 CARDIAC β-ADRENERGIC RECEPTOR BINDING CHANGES WITH AGE IN RHESUS MONKEY BUT NOT IN RAT. Barton G. Weick and Sue Ritter. Dept. of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164.

Mid-left ventricular wedges were collected from 14 rhesus monkeys (4, 8, 13, 23, 24 and 31 years old) during planned sacrifice under pentobarbital anesthesia. In addition, whole hearts were collected from 24 rats (5, 12, 19 and 27 months old). Using ^{3}H-dihydroalprenolol, the density of cardiac β-adrenergic receptors and the apparent K_D of the radioligand were determined by Scatchard analysis. Although β-receptor density was 86% greater in rhesus monkey left ventricular wedges than in whole rat hearts, receptor density did not change with age in either rats or monkeys. When K_D values were averaged across all samples, no species differences in K_D were observed (K_D=2.63 nM and 2.54 nM for rats and monkeys, respectively). Furthermore, as reported previously, the apparent K_D of cardiac β-receptors did not vary with age in rats. In contrast to rats, however, the apparent K_D was positively correlated with age in rhesus monkey heart samples ($r=.808$, $p<.001$, K_D=[.00602 x age in months]+1.349). The apparent K_D more than doubled across age groups, ranging from 1.70±.09 nM in the 4 yr old monkeys ($n=3$) to 3.71±.61 nM in the 31 yr olds ($n=3$).

These results indicate that the affinity of cardiac β-adrenergic receptors for receptor ligands significantly decreases with age in rhesus monkeys but not in rats. Therefore, these data suggest that the rat may not provide a satisfactory model for the study of age-related changes in cardiac β-receptors in primates, including humans.

74.10 CHANGES IN BODY TEMPERATURE, LH SECRETION AND HYPOTHALAMIC CATECHOLAMINERGIC NEURONAL ACTIVITY DURING MORPHINE WITHDRAWAL IN FEMALE RATS. S.M. Gabriel*, I. Song*, M.J. Katovich* and J.W. Simpkins* (spon: W.G. Luttge). College of Pharmacy, University of Florida, Gainesville, FL 32610.

The tail skin temperature (TST) instability seen during the morphine withdrawal in the rat may serve as a useful animal model for opioid participation in the perimenopausal hot flush. Since "hot flushes" in women are consistently accompanied by surges of Luteinizing Hormone (LH) secretion, the present study investigated whether TST fluctuations in morphine withdrawn animals are associated with surges of LH secretion, and if these changes are, in turn, accompanied by altered hypothalamic catecholamine metabolism. Morphine (MOR) dependency was induced by multiple implantation of pellets containing 75mg MOR freebase, and animals were withdrawn by s.c. injection of naloxone HCl (1.0 mg/kg NAL). Ovariectomized, estrogen replaced rats received MOR for 4 days, then TST was recorded at 5 minute intervals before and for 90 minutes after NAL injection. Blood samples were withdrawn through jugular catheters prior to and 5, 10, 15, 30 and 60 minutes after NAL. NAL induced a 13-fold increase in LH within 10 minutes ($p<0.001$) which preceded by 5 minutes a 4.3 ± 0.2°C peak elevation in TST ($p<0.001$).

In a second study, rats were ovariectomized and immediately treated with MOR. After 3 days groups of rats were sacrificed 0, 5, 15, 30 and 60 minutes after NAL. Serum LH increased by 9-fold (11.3 ± 3.2 to 99.1 ± 23.6 ng/ml) within 5 minutes of NAL treatment and remained elevated at least 60 minutes. The activity of catecholamine neurons was generally enhanced during withdrawal. Increases of 166 to 237% in the norepinephrine (NE) metabolite, normetanephrine (NME), were seen by 15 minutes after injection of NAL in both the medial basal hypothalamus (MBH) and the preoptic area (POA). This was followed by 25 to 40% decreases in NE by 30 to 60 minutes after NAL precipitated withdrawal. Concentrations of dopamine (DA) and its metabolite, dihydroxyphenylacetic acid (DOPAC), increased in both areas by 30 minutes ($p<0.05$) following NAL administration. A second DA metabolite, homovanillic acid (HVA), increased 2-fold in concentration in the MBH 5 minutes after naloxone induced withdrawal, but was unchanged throughout this period in the POA. These studies demonstrate that TST fluctuations in morphine withdrawn animals are temporally associated with surges in LH secretion, supporting the usefulness of this animal model for the perimenopausal hot flush. Further, these rapid changes in TST and LH secretion may be mediated by increased catecholamine metabolism which follows the precipitous withdraw from morphine. (Supported in part by NIH AG02021 to JWS).

74.11 A VERIFIABLE ANIMAL MODEL OF GLOBAL CEREBRAL ISCHEMIA. A. S. Hernandez,* A. Benedetto,* M. L. Lecklitner,* M. S. Albin, M. L. Nusynowitz,* and L. Bunegin.* Neuroanesthesia and Nuclear Medicine Labs., Depts. of Anesthesiology and Radiology, Univ. Tx. Hlth. Sci. Ctr., San Antonio, TX 78284.

Experimental global cerebral ischemia (GCI) has been produced using aortic cross clamping, high cisterna magna pressures, balloon occlusion of inflow/and outflow vessels to brain, chemical and electrical cardiac arrest, decapitation and a high pressure neck tourniquet (HPNT). An ideal GCI model should be non-invasive; in a non-primate, mammalian,inexpensive animal; where the cerebral circulation can be easily reestablished after termination of GCI; and where GCI could be easily verified in every member of the experimental population. The HPNT technique using the rat comes close to meeting all these criteria. In the past, HPNT induced GCI has been hampered by the difficulties involved in definitively assuring lack of blood flow to brain during ischemia time. The present study demonstrates a simple method to obtain verification of GCI.

Long-Evans rats (350-500g) were anesthetized with 25 mg/kg of thiopental intraperitoneally. A tail vein was cannulated with a 25 gauge infusion set needle and succinylcholine, 1.0 mg was given intravenously to facilitate intubation with an 18 gauge plastic catheter. Each rat was placed on a Harvard rodent ventilator on room air at 4.0-5.0 ml. tidal volume and a respiratory rate of 50/min. These respirator settings maintain a $PaCO_2$ of 35-50 torr with a PaO_2 greater than 90 torr. The rat was then placed under a scintillation camera with computer aquisition of static and dynamic data. A specially designed inflatable tourniquet was placed tightly about the neck of the anesthetized rat and inflated to 60 psi with the total period of ischemia being 14 minutes. Acute GCI was documented in all 10 animals tested by the tail vein injection of five mCi of technetium-99 m with concurrent data aquisition nine minutes after tourniquet inflation. Data aquisition was continued for an additional five minutes of ischemia and for an additional 10 minutes following deflation of the tourniquet. Using this technique, total occlusion of the cerebral circulation can be assured and those animals in which GCI was not present can be rejected. This inexpensive, non-primate model can become a useful tool in evaluating the physiopathology of GCI as well as looking at pharmacological agents that may have the possibility of attenuating the deleterious responses. Supported in part by NIH grant RR 05654.

74.12 PREDISPOSITION TO GASTRIC LESIONS IN GENETICALLY OBESE (OB/OB) MICE: A CONSEQUENCE OF AUTONOMIC DYSFUNCTION. D. Greenberg * and S. H. Ackerman* (SPON: M. A. Hofer). Hunter Col. of C.U.N.Y. New York, N.Y. 10021,*and Dept. of Psychiatry, Montefiore Hosp. and Med. Ctr. Albert Einstein Col. of Med. Bronx N.Y. 10467.

Genetically obese (Ob/Ob) mice are, in contrast to lean littermates, unable to maintain normal body temperature when placed in a cold environment. In the rat, physical restraint interferes with body temperature regulation and, as a consequence, produces gastric erosions (Ackerman et al. Sci.,201: 373, 1978). We therefore tested the hypothesis that Ob/Ob mice would be more susceptible to restraint induced gastric erosions than their lean littermates.

Fifteen Ob/Ob mice and 15 lean littermate control animals were food deprived for 24 hours at 20 °C and subsequently restrained in wire mesh cones for 24 hours at 20 °C. During this procedure oxygen consumption was measured. Body temperature was determined prior to and following food deprivation, and following restraint. At autopsy stomachs were examined for erosions. Wet weight of inguinal, epidydimal and retroperitoneal white adipose tissue and interscapular brown adipose tissue was determined.

Ob/Ob mice were found to have significantly more gastric erosions than did lean littermates (47% as opposed to 7% incidence, $p<.05$). Ob/Ob mice became hypothermic during food deprivation and restraint, while lean animals were able to maintain body temperature under these conditions. This was true even though the weights of white adipose tissue deposits were much greater in obese animals and thus heat loss would be substantially decreased due to greater insulation. Ob/Ob mice were found to use significantly less oxygen during all phases of the experiment. Thus restraint at room temperature induces hypothermia that appears associated with insufficient heat production. Although Ob/Ob mice have known deficiencies in brown adipose tissue mediated non-shivering thermogenesis in cold environments, this may not adequately explain their deficit in heat production during food deprivation and restraint. Preliminary results from the use of β adrenergic agonists during restraint show a paradoxical decrease in oxygen consumption in Ob/Ob mice. Lean animals show the typical increase in oxygen consumption when given these agents. These results suggest that in restraint Ob/Ob mice have autonomic disturbances other than those involved with brown fat metabolism.

We conclude that Ob/Ob mice are more susceptible to restraint induced gastric erosions than their lean littermates. This susceptibility is associated with a disturbance in heat production and a consequent failure of body temperature regulation during food deprivation and restraint.

75.1 SELECTIVE HEPATIC VAGOTOMY BLOCKS THE SATIETY EFFECT OF PANCREATIC GLUCAGON. N. Geary* and G.P. Smith. Dept. Psychiatry, Cornell Univ. Med. Coll., White Plains, NY 10605

Peripheral injection of pancreatic glucagon before meals produces an immediate, specific inhibition of feeding in the rat. Martin et al. (Am. J. Physiol. 234:E314, 1978) demonstrated that total abdominal vagotomy blocked glucagon's satiety effect and hypothesized that hepatic vagal afferents mediate the effect. To test this, we investigated the effects of selective lesions of the abdominal vagus on glucagon-induced satiety. Glucagon (100 - 400 µg/kg) was intraperitoneally injected just before presentation of a palatable liquid diet to 3 h food deprived rats with selective vagotomies of only the hepatic branch of the abdominal vagus, with selective vagotomies of the entire abdominal vagus except the hepatic branch, or with complete abdominal vagotomies. Hepatic vagotomy or total vagotomy completely blocked glucagon's satiety effect, but vagotomy that spared the hepatic branch did not significantly change the satiety effect in comparison to sham-operated controls. These results indicate that hepatic vagal fibers are the necessary and sufficient contribution of the abdominal vagus to glucagon's satiety effect. This is consistent with, but does not prove, the hypothesis that increased glucagon receptor binding in the liver produces a satiety signal that is relayed to the brain over the vagus nerve.

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75.2 VAGOTOMY BLOCKS INTESTINAL SATIETY. J. Gibbs, G.P. Smith and H. Derry*. Dept. of Psychiatry, Cornell Univ. Med. Coll. and E.W. Bourne Lab., The New York Hospital, White Plains, NY 10605.

After an overnight food deprivation, rats sham feed continuously when all of an ingested liquid food drains from an open gastric cannula (Young et al, 1974). Under these conditions, if small amounts of liquid food are infused directly into the duodenum through a chronic indwelling catheter, sham feeding stops and the sequence of behaviors characteristic of normal satiety appears (Liebling et al, 1975). To determine if this 'intestinal satiety' depends on vagal nerve supply, we tested the ability of intestinal food infusions to stop sham feeding in vagotomized rats.

Method: Nine male Sprague Dawley rats (400 - 500 g body weight) received bilateral total abdominal vagotomies. Two rats underwent sham vagotomies and served as controls. After a two-week recovery period, all rats were equipped with (1) chronic stainless steel gastric cannulas which could be temporarily opened to allow sham feeding, and (2) chronic Silastic catheters, anchored in the second portion of the duodenum, through which liquid food could be infused. After recovery from the second operation, the rats were accustomed to an overnight (1700-1000) food deprivation followed by a daily 60 min sham feeding test during which they consumed a balanced, diluted liquid food (25% BioServ, Frenchtown, NJ). Twelve minutes after sham feeding began, each rat received an intestinal infusion of 25% liquid food or 0.15M NaCl as a control. The volume of infusate was 6 ml, delivered at a rate of 0.5 ml/min.

Results: Vagotomized rats completely failed to suppress intake during intestinal infusions of liquid food: on days when food was infused, they sham fed 69.5 ± 13.2 ml (mean ± SEM) during the 30 min which followed the beginning of the infusion; on days when saline was infused, they sham fed 57.0 ± 10.0 ml. Adequacy of vagotomy was confirmed by failure to drink following hypertonic NaCl and/or by anatomical inspection at autopsy. In contrast, duodenal infusion of food into the 2 sham vagotomized rats inhibited sham feeding significantly ($p<.05$).

Conclusion: These results demonstrate that the abdominal vagus is necessary for the satiety effect of food in the small intestine.

This study was supported by USPHS grants AM17240, MH15455, MH00149, and the Irma T. Hirsch Foundation.

75.3 GLUCOPRIVIC FEEDING OCCURS IN THE ABSENCE OF REDUCED GLUCOSE OXIDATION. V.K. Nonavinakere 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.

We have previously reported that feeding in response to either systemic or intracerebroventricular administration of 2-deoxy-D-glucose (2DG) persists 6 to 8 hours post 2DG injection when other physiological responses to glucoprivation have abated. This delayed feeding suggests either that ongoing glucoprivation is not necessary for feeding or that responses such as hyperglycemia abate while glucoprivation is still extant and able to stimulate feeding.

In order to determine whether glucoprivation is still present after the sympathoadrenal hyperglycemic response to 2DG has abated, we measured glucose oxidation in rats treated with 2DG 0.5 hr or 6 hrs prior to respirometric experiments. We found that glucose oxidation was reduced by 67% ($P<.02$) during the first 3.5 hrs post 2DG. However, glucose oxidation of 2DG-treated rats was indistinguishable from that of controls by 6 hrs post 2DG administration. The rate of glucose disposal from plasma of 2DG-treated rats did not differ from that of saline injected controls either during the first 3.5 hrs or 7-9 hrs post 2DG administration.

In order to determine whether hexose accumulation by the brain is impaired by 6 hrs post 2DG, we measured regional brain ^{14}C-2DG uptake in rats treated with 2DG (200 mg/kg) or saline 0.5 hr or 6 hrs prior to injection of ^{14}C-2DG. We found that the uptake of ^{14}C-2DG into the brain was significantly reduced ($P<.001$) 1.5 hr post 2DG but was not impaired by 7 hrs post 2DG administration.

These results suggest that ongoing reduction of systemic glucose oxidation and ongoing impairment of hexose availability to the brain need not occur during glucoprivically induced feeding.

75.4 ATTENUATION OF CHOLECYSTOKININ-INDUCED SATIETY IN RATS PRETREATED WITH CAPSAICIN. R.C. Ritter and E.H. South. 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.

Section of the gastric vagus abolishes suppression of food intake brought about by cholecystokinin (CCK) (Smith et al., Science, 213:1036-1037, 1981). This finding suggests that vagal afferents may mediate the satiety-producing effect of CCK. However, surgical vagotomy does not rule out a role for vagal efferents in the CCK effect. Furthermore, vagotomy cannot implicate a specific population of afferents in the mediation of CCK satiety.

Capsaicin, the pungent principle of red pepper, damages fine, unmyelinated, peptidergic afferent neurons in the vagus nerve and elsewhere. Efferent neurons and large diameter afferents are not affected by capsaicin. In order to determine whether capsaicin-sensitive neurons participate in the expression of CCK-induced satiety, we tested capsaicin-pretreated and control rats for suppression of food intake in response to intraperitoneal (IP) injections of CCK. Adult, male rats, anesthetized with Metofane, were given two IP capsaicin injections (25 mg/kg and 50 mg/kg) 4 hrs apart. The rats received a third capsaicin dose (50 mg/kg) 24 hr after the first injection. Control rats received equivolumetric vehicle injections. By 2 wks post-injection, body weights and 24 hr food intakes of capsaicin injected rats were not different from those of controls. When offered a preferred food (cookies), capsaicin-treated rats and controls ate statistically indistinguishable amounts. However, after IP CCK control rats ate significantly less than capsaicin treated rats at all CCK doses (see table). In fact, CCK did not significantly reduce intake in capsaicin treated rats except at a dose of 4 µg/kg.

	CCK Dose (µg/kg)			
	0	1	2	4
Capsaicin-Treated (N=10)	4.7±0.8*	4.3±0.4	3.5±0.4	2.8±0.7
Vehicle-Treated (N=9)	4.3±0.7	2.7±0.5	1.3±0.3	1.4±0.1

*Food intake in grams/30 min ± SEM

A ten ml intragastric preload of liquid diet suppressed food intake similarly in both groups.

The results indicate that capsaicin-sensitive, afferent neurons participate in mediation of CCK-satiety. The fact that capsaicin treatment does not totally abolish sensitivity to CCK may mean that our treatment did not inactivate all capsaicin sensitive cells. Alternatively, residual CCK sensitivity may indicate multiple receptor mechanisms for CCK-satiety.

75.5 INFLUENCE OF INTRAVENTRICULAR INSULIN ON HYPOTHALAMIC UNIT ACTIVITY AND DEPRIVATION-INDUCED FEEDING. E.K. Walls* and T.B. Wishart. Psychology Dept., Univ. of Saskatchewan, Saskatoon, Sask., Canada, S7N 0W0.

Increased appetite in humans and acute hyperphagia in experimental animals are typically observed in response to insulin-induced hypoglycemia. As large doses of insulin are required to reliably produce hyperphagia and it is known that, when administered intracisternally, insulin does not pass the cerebrospinal fluid (CSF) blood-brain barrier (Chowers, I., Lavy, S. & Halpern, L. Exp. Neurol., 3:197, 1961), the aim of the present study was to determine if eating could be influenced by locally elevating the CSF insulin level in the rat.

Chronic access to the third ventricle was provided by a single cannula (feeding experiments) or dual cannulae (recording experiments) as described in (Walls, E.K. & Wishart, T.B. Physiol. Behav., 19:171, 1977). In 21 hour food-deprived rats, intraventricular injection of regular insulin (600 mU) 30 minutes prior to food presentation produced a significant elevation in food intake above saline control levels. Intraperitoneal administration of this dose of insulin did not affect food intake. The insulin-induced elevation in food intake was not altered by intraventricular injection of the glucose uptake blocker phloridzin (3μg) but was reduced by the α-adrenergic antagonist phentolamine (15 μg). When insulin was administered in combination with the β-adrenergic antagonist propranolol (60μg), the elevation in food intake was blocked.

The activity of lateral hypothalamic neurons was recorded during intraventricular injections of insulin and adrenergic antagonists. All neurons that were sensitive to phenotolamine responded in an opposite direction following insulin treatment. Although neurons found to be sensitive to propranolol also responded to insulin treatment a differential response pattern was not observed.

When ^{125}I-labelled insulin was injected into the third ventricle it was not detected in the blood within four hours of injection. The finding that the elevated feeding response to intraventricular insulin occurred in the absence of changes in blood glucose further suggests that this effect is centrally mediated and is unrelated to peripheral hypoglycemia. The parallel observations that centrally administered α- and β-adrenergic antagonists affect insulin-enhanced food intake and influence the activity of insulin-sensitive neuronal elements in the lateral hypothalamus, suggest insulin may interact with central adrenergic mechanisms in the control of food intake in the rat.

75.6 GENETICALLY OBESE ZUCKER RATS HAVE INAPPROPRIATELY LOW IMMUNO-REACTIVE INSULIN LEVELS IN CEREBROSPINAL FLUID. L.J. Stein*, D.L. Hjeresen, D. Porte, Jr.* and S.C. Woods. Dept. of Psychology, Univ. of Washington and Seattle Veterans Administration Research Center, Seattle, WA 98195.

We have proposed that insulin within the cerebrospinal fluid (CSF) provides a negative feedback signal to the central nervous system in the regulation of body adiposity by food intake. According to this hypothesis, an increase of body adiposity is associated with an increase of insulin secretion from the pancreas, which is in turn reflected as an elevation of CSF insulin. The increased CSF insulin interacts with areas of the brain influencing food intake such that meal size is reduced until body weight returns to its normal level. The opposite would occur given a decrease of body adiposity.

Although this hypothesis is supported by considerable circumstantial evidence, only one study to date has examined directly the relationship between body adiposity and CSF insulin levels. Owen et al. (Metab. 23:7, 1974) reported that obese humans have higher CSF insulin than lean controls. We have examined plasma and CSF immunoreactive insulin (IRI) in genetically obese Zucker rats, their lean controls, and normal Long-Evans rats. Animals were anesthetized and CSF samples taken from the cisternum magnum. Blood samples were then taken via cardiac puncture.

Fatty Zuckers weighed 652 $\pm$ 21 g (M $\pm$ SEM) and lean Zuckers weighed 355 $\pm$ 17 g ($p < .001$). Plasma IRI for the obese and lean Zuckers were 4.9 $\pm$ 0.7 and 2.7 $\pm$ 0.6 ng/ml, respectively ($p < .05$). However, CSF IRI was not significantly different between the two groups (Obese = 0.12 $\pm$ 0.05; leans = 0.09 $\pm$ 0.03 ng/ml), and the ranges of the two groups were virtually identical (Obese = 0.04 to 0.28; leans = 0.04 to 0.26 ng/ml). Plasma IRI of the Long-Evans rats averaged 1.7 $\pm$ 0.2 ng/ml and CSF IRI was 0.01 $\pm$ 0.01 ng/ml. Neither measure differed significantly from the analogous values for the lean Zuckers.

The finding that obese Zucker rats have normal CSF IRI in spite of increased plasma IRI suggests that they might have a deficit in the system which transports insulin from the plasma to the CSF. Their obesity might therefore result, at least in part, from an inability to convey accurate information concerning adiposity from the periphery to the brain.

75.7 BRAIN INSULIN CONCENTRATIONS IN HYPERINSULINEMIC FATTY ZUCKER RATS ARE NOT ELEVATED. D.G. Baskin* and D.M. Dorsa* (SPON: W. Stahl). Depts. of Biological Structure, Pharmacology, and Medicine, Univ. of Washington School of Medicine, Seattle, WA 98195, and VA Medical Center, Seattle, WA 98108.

Recent evidence indicates that insulin of pancreatic origin may be a feedback signal to the central nervous system for the long-term regulation of feeding and body weight. This suggests that obesity in some animals may be related to restricted access of insulin to the central nervous system and predicts that obese rats which have high plasma insulin levels may have inappropriately low brain insulin content. In order to test this hypothesis, we measured the concentration of immunoreactive insulin (IRI) extracted from several regions of the brain of fasted hyperinsulinemic, obese, female Zucker rats (ZF) and their lean littermates (ZL) and compared these with normal Wistar rats (W). Brains obtained after decapitation were dissected into hippocampus (HI), amygdala (AM), cerebral cortex (CC), midbrain (MB), and hindbrain (HB), which were frozen on CO_2 ice. Insulin was extracted from the fragments by homogenization in 0.2M HCl/75% ethanol at 0°C, and IRI was measured by radioimmunoassay. The results (ng/g wet weight) compared to fasting plasma (PL) levels (ng/ml) of IRI were:

		HI	AM	CC	MB	HB	PL	B/PL RATIO
W	$\bar{x}$	.27	.20	.26	.20	.24	1.20	.20
	SEM	.04	.04	.04	.02	.01	.35	
	N	4	4	3	4	5	8	
ZL	$\bar{x}$	.08	.10	.07	.06	.08	.32	.24
	SEM	.01	.02	.03	.01	.02	.10	
	N	6	6	6	6	6	6	
ZF	$\bar{x}$	.08	.09	.06	.06	.08	1.84	.04
	SEM	.01	.02	.02	.02	.02	.25	
	N	6	6	6	6	6	6	

There was no difference in brain IRI levels between Zucker Lean and Zucker Fatty rats, even though the Zucker Fatty rats had 6 times greater plasma IRI concentrations than Zucker Lean rats. Although brain IRI concentrations in Zucker Lean and Zucker Fatty rats were lower than in Wistar rats, the brain-to-plasma IRI ratio (B/PL) was similar for both Wistar and Zucker Lean rats. In contrast, the B/PL IRI ratio was much lower in Zucker Fatty rats. These results suggest that in Zucker Fatty rats, proportionally less plasma insulin enters the brain than in Zucker Lean rats. We propose that reduced entry of plasma insulin into the central nervous system may contribute in part to hyperphagia and obesity in Zucker Fatty rats. (Supported by NIH AM 17047 and the Veterans Administration.)

75.8 VAGAL AFFERENTS TO THE AREA POSTREMA/CAUDAL-MEDIAL NUCLEUS OF THE SOLITARY TRACT IMPORTANT FOR FOOD INTAKE AND BODY WEIGHT: AN HRP STUDY. R. Eng, R.E. Shapiro*, and R.R. Miselis. Dept. Anat., Sch. Vet. Med., Univ. Penn., Philadelphia, PA 19104.

The vagus nerve is involved in food intake and the regulation of body weight in normal rats (Mordes et al. 1977; Eng et al. 1979) and is essential for hypothalamic hyperphagia and obesity (Powley and Opsahl, 1974; Sawchenko and Gold, 1981). Until recently, the role of motor components of the vagus nerve was emphasized in the latter phenomenon. However, lesions of the area postrema/caudal-medial nucleus of the solitary tract (AP/cmNTS) sparing the dorsal motor nucleus of the vagus (DMN) reverse hypothalamic knife cut hyperphagia and obesity with no motor deficits in intestinal transit and gastric retention (Hyde, Eng, and Miselis, 1981). AP/cmNTS lesions also affect ingestion and body weight regulation in the normal rat (Hyde and Miselis, in press, 1982). The present report provides neuroanatomical evidence for the hypothesis that the AP/cmNTS lesion exerts its effect via an interruption of vagal afferent input from the abdomen. In male albino rats the subdiaphragmatic vagal trunks were exposed via a ventral incision and incubated for 2-4 hrs. in free HRP crystals (Sigma VI) moistened with isotonic saline. After a 72 hr. survival the brains were removed and processed using a TMB protocol (Mesulam, 1978). Retrogradely labelled cells were seen in the DMN, nucleus ambiguus, and nodose ganglia. The sensory fibers entered the rostrodorsal medulla and descended in the solitary tract to their projection fields primarily in the medial and caudal-medial NTS. A fine-grained reaction product characteristic of terminals was found in the medial NTS. A crescent shaped area in the commissuralis portion of the NTS ventral to the AP was particularly heavily labelled. Our AP/cmNTS lesion damage correlated well with the labelled area in the commissural NTS. The present anatomical results confirm and extend previous studies (Coil and Norgren, 1979; Kalia and Mesulam, 1980; Leslie et al., 1982). Taken together our anatomical and behavioral data support the notion that food intake and the maintenance of body weight is partially dependent on abdominal sensory input. Furthermore the AP/cmNTS is directly and reciprocally connected with the hypothalamus (Shapiro and Miselis, unpublished; Ricardo and Koh, 1978; Koh, 1981; Sawchenko and Swanson, 1981), especially with the paraventricular nucleus which has been implicated in hyperphagia and obesity (Gold et al., 1977; Eng, Nunez, and Gold, 1979; Eng and Gold, in preparation; Leibowitz, 1979, 1981). Therefore the AP/cmNTS because of its central and peripheral connections and its lack of a blood-brain barrier is a focal point of information convergence crucial for ingestive behavior and body weight regulation. (GM27739 and MH08742)

75.9 CHOLECYSTOKININ (CCK) INFUSED INTRAPERITONEALLY DURING EACH MEAL FOR SIX DAYS CHRONICALLY SUPPRESSES MEAL SIZE AND INCREASES MEAL FREQUENCY OF FREE-FEEDING RATS, D.B. West, D. Fey* and S.C. Woods. Depts. of Physiology and Psychology, University of Washington, Seattle, WA 98195

CCK has been demonstrated to suppress the size of single meals in mildly deprived rodents, yet this putative satiety action has not been studied in free-feeding rodents over an extended time period. In order to address the effectiveness of chronic CCK injection, 5 male Long Evans rats were habituated for ten days to individual computer controlled cages which continuously monitored both food and water intake. Over a 5-Day baseline period, daily food intake averaged 24.52 g (± 0.47) with a meal frequency of 11.88 meals/day (± 1.36) and an average meal size of 2.18 g (± 0.25). During this baseline the rats were gaining an average of 3.15 g (± 0.54) each day. These rats were then implanted with indwelling intraperitoneal catheters and attached to computer controlled remote infusion pumps programmed to dispense 0.27 ml of physiological saline at the start of each meal. By the fourth day following surgery, growth rate had returned to approximately its presurgical level (3.30 ± 0.49 g/day). Beginning on the fifth post-surgical Day, CCK was infused during each meal at a dose of 1.1 microgram/rat. On the first day of CCK infusion, total daily intake was significantly reduced to 14.38 g (± 0.58), meal size was reduced to 0.72 g (± 0.027) and meal frequency increased to 19.8 meals/day (± 1.16). All of these pattern shifts were statistically significant when compared to baseline values by Analysis of Variance ($p \leq .05$). After 6 days of CCK infusion total daily intake was no longer reduced relative to baseline (23.07 ± 0.68 g); however meal size remained suppressed (1.20 ± 0.14 g) and meal frequency was elevated (19.6 ± 1.75 meals/day) ($p \leq .05$, Analysis of Variance). Body weight dropped an average of 12.4 g after one day of CCK infusion but the growth rate over the next 5 days of drug infusion remained the same as that during baseline conditions. With the cessation of CCK infusion, meal frequency immediately returned to baseline (13.2 ± 0.37 meals/day) and meal size also returned to pre-CCK levels (2.13 ± 0.20 g). These data demonstrate conclusively that tolerance to the meal size suppressive action of CCK does not develop when it is administered prior to each meal. These findings also indicate that, although chronic CCK administration will result in smaller meals, the rat will defend its body weight and rate of growth by modifying meal frequency to ensure adequate nutrition.

75.10 REGULATION OF FEEDING BY BLOOD BORNE HUNGER AND SATIETY SUBSTANCES THROUGH GLUCOSE-SENSITIVE NEURONS. Y. Oomura, N. Shimizu*, A. Inokuchi*, T. Sakata*, K. Arase*, M. Fujimoto*, M. Fukushima* and K. Tsutsui*. Dept. of Physiology, Kyushu Univ., Fukuoka 812, Japan.

Since the contribution of endogenous factors as physiologically effective signals of hunger and satiation, and the elicitation and termination of feeding behavior still remain uncertain, we have examined the effects of newly found organic acids, in the blood of fasted rats during various hunger stages. The levels of 2-deoxytetronate (2-DTA) $CH_2(OH)\cdot CH(OH)\cdot CH_2\cdot COOH$, 3-deoxytetronate (3-DTA) $CH_2(OH)\cdot CH_2\cdot CH(OH)\cdot COOH$, and 3-deoxypentonate (3-DPA) $CH_2(OH)\cdot CH(OH)\cdot CH_2\cdot CH(OH)\cdot COOH$, increase gradually and peak at around 36 hr during fasting (Sakata et al., Brain Res. Bull. 5, Suppl. 4, 23, 1980; Oomura, Japan J. Physiol. 31 Suppl. 1, 1981).

After one shot application of 2-DTA into 3rd cerebral ventricle in normal rats at 1μ mole concentration, food intake was suppressed continuously for 24 hr on all tested rats. The suppressive effect was dose-dependent. On the other hand 3-DTA transiently elicited feeding, in one fifth of the rats, while 3-DPA elicited short-lasting food intake with prandial drinking of water in two thirds of the rats tested, with a latency of 10-20 min. Control 0.15 M NaCl had no effect. Bilateral osmotic minipump infusion of these substances for one week into the lateral hypothalamic area (LHA) resulted in similar effects on the amount of food intake, i.e. 2-DTA suppressed while 3-DPA increased. Single LHA neural discharges recorded by chronically implanted Pt wire microelectrodes were continuously suppressed for more than 2 hr by one shot application of 2-DTA into the 3rd ventricle in 24 hr food deprived rats accompanied by food intake suppression. On the contrary the neural discharges were increased on application of 3-DPA with 10-20 min latency accompanied by elicitation of food intake. Iontophoretically applied 2-DTA immediately depressed significantly and selectively glucose-sensitive neuronal activity in the LHA of anesthetized rats, 3-DTA did not inhibit these neurons, while 3-DPA caused excitation significantly on the glucose-sensitive neurons, thus suggesting new aspects of molecular size dependent structure specificity by LHA neurons. These substances did not have any effect on the glucose insensitive neurons.

Due to a high correlation between activity of LHA glucose-sensitive neurons and feeding behavior, and consistency of the change in long-term chronic LHA neuronal activity by microinjection of the organic acids, it is postulated that 2-DTA is an endogenous hunger substance and 3-DPA a satiety substance, and these effects are mediated through the glucose-sensitive neurons in the LHA.

75.11 ALTERATIONS IN WATER BALANCE AND RESPONSIVENESS TO REGULATORY CHALLENGES IN RATS WITH LESIONS OF THE AP/cmNTS. Thomas M. Hyde and Richard R. Miselis. Inst. Neur. Sci., Animal Biology, Sch. Vet. Med., Univ. of Penn., Philadelphia, PA, 19104.

The area postrema (AP) and the contiguous caudal nucleus of the solitary tract (cmNTS) are the sites of termination of subdiaphragmatic vagal sensory afferents. Ablation of the AP/cmNTS eliminates abdominal visceral sensory input to the CNS, and causes a syndrome of transient hypophagia and permanent reduction in body weight. Although there is an absolute hypodipsia for the first 12 post-op days, there is a relative polydipsia with respect to food intake. By the 16th post-op day, lesioned rats display an absolute hyperdipsia which persists for at least 150 days, and is accompanied by elevated water/food ratios. Three weeks post-op, after 22 h. of water deprivation, lesioned rats drink 76% more water than controls in a 6 h. test, (7.5±0.5 ml/100 g BWt vs. 4.2±0.2). Under total deprivation conditions, 24 h. urine function tests were performed. Immediately post-op, lesioned rats excrete 12.7±1.2 ml urine vs. 9.3±0.5 ml for controls, a difference of 38%. The lesioned rats lose 0.128±0.016 meq Na^+ vs. 0.072±0.020 for the controls. This suggests that the AP/cmNTS lesion causes a primary diuresis and natriuresis. Twenty-three weeks post-op, using the same paradigm, lesioned rats excrete 79.3% more urine (per 100 g BWt) than controls, showing the persistence of this deficit. Three weeks post-op, lesioned and control rats were given access to water and 3.0% NaCl solution. Over a 24 h. test, lesioned rats drink 6.0±2.0 ml water/100 g BWt while controls drink 2.4±0.9 ml. The lesioned rats also consume 5.1±2.0 ml 3.0% NaCl/100 g BWt vs. 0.3±0.2 for controls. This suggests that the lesioned rats compensate for their increased urinary water and sodium losses through increased ingestion. Finally, both lesioned and control rats were tested 7 weeks post-op with a SC injection of 2 M NaCl (0.33 ml/100 g BWt), and water intake was recorded over the next 24 h. One h. after the injection lesioned rats drink 7.5±0.9 ml over baseline vs. 3.9±0.8 for controls. At six h., the relative intakes are 12.8±1.8 ml vs. 7.5±1.1 ml. However, at the end of the 24 h. test period, the lesioned rats consume the same amount of water over baseline levels as the controls, suggesting delayed behavioral compensation. Loss of the AP/cmNTS causes rats to over-compensate behaviorally and to underconserve renally.

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75.12 VAGOTOMY PRODUCES LEARNED FOOD AVERSIONS IN THE RAT. I.L. Bernstein and L.E. Goehler.* Dept. Psychol. Univ. of Washington, Seattle, WA 98195.

An important role for the vagus nerve in the control of appetite has been suggested by studies examining the effects of vagotomy on food intake. For example, subdiaphragmatic vagotomy produces hypophagia and weight loss in normal rats and reverses the hyperphagia and obesity of VMH-lesioned rats. The clinical literature also describes symptoms of nausea and discomfort after meals ("dumping syndrome") in humans with vagotomies. Since these symptoms are highly effective as unconditioned stimuli in food aversion conditioning the present study examined whether some of the depression in food intake observed in rats with vagotomy could be due to the development of aversions to foods eaten after their surgery.

Wistar rats with bilateral subdiaphragmatic vagotomies (VAG: n = 14) and sham-operated controls (CON: n = 12) were given continuous access to one of two nutritionally adequate diets (AIN or C-21) for nine days. Food intake during this period was considerably lower for vagotomized animals (VAG-AIN = 5.8g; VAG-C-21 = 5.2g) than for controls (CON-AIN = 19.6g; CON-C-21 = 16.4g) ($p < .01$). On the tenth day a preference test was administered in which animals had access to both AIN and C-21 diets for four hours. Results of this test were that vagotomized groups showed significantly lower preferences than controls for the specific diet they had consumed during the diet exposure period ($p < .01$), indicating that the symptoms associated with vagotomy can serve as effective unconditioned stimuli in the acquisition of learned food aversions. Additional preference tests, administered in subsequent weeks, indicated that learned food aversions in vagotomized animals persist for several weeks. Furthermore, when a 'non-aversive' food was offered to vagotomized groups their food intake rose immediately.

Results of this study indicate that learned food aversions can contribute to the appetite and weight loss exhibited by vagotomized animals. Consideration of the conditions under which learned food aversions arise after vagotomy surgery may allow for the design of studies so as to minimize them and thereby separate these non-specific effects from direct regulatory deficits produced by vagotomy. (Supported by USPHS Grant CA26419).

75.13 INSULIN ELICITS INGESTION IN CHRONIC DECEREBRATE RATS.
Francis W. Flynn*and Harvey J. Grill, Dept. of Psychology & Inst. Neurol. Sci., U. of Pennsylvania, Philadelphia, PA 19104

Forebrain structures are thought to control both autonomic and behavioral responses necessary for energy homeostasis. Recently the hegemony of the forebrain has been questioned by studies on forebrain-ablated rats. Chronic decerebrate rats demonstrate the sympathoadrenal response following 2DG injection (DiRocco & Grill 1979) and increase their ingestion of sucrose solution as a function of food deprivation (Grill & Norgren 1978). The capacity of the caudal brainstem to control ingestive consummatory responses following insulin injections was examined in supracollicular-transected and control rats. Rats were decerebrated in two stages using a hand-held spatula. Both control and chronic decerebrate rats were fitted with intraoral fistulae. Rats were tube-fed three 12-ml meals daily. Following their morning meal, rats were injected subcutaneously with either saline (0.9%), 5U/kg or 10U/kg regular insulin. Tail blood samples were taken hourly thereafter for 6 hrs. Insulin produced a marked hypoglycemia in both chronic decerebrate and control rats. The nadir of the hypoglycemia was at 3-4 hrs following the injection for both groups. Based on this result, intake was examined at 3 hrs post-injection. Chronic decerebrate and control rats were comparably glycemic at the time of the intake test in both saline (control=140 mg%; decerebrate=137 mg%) and insulin (5U/kg control=48 mg%, decerebrate=70 mg%; 10U/kg: control=52 mg%, decerebrate=56 mg%) injection conditions. Protocol: Rats were tubed their morning meal; 1 hr later injected (saline, 10U/kg or 5U/kg, sc), and tested 3 hrs following injection. During the intake test either .03 M sucrose solution or distilled water was infused intraorally until the solution was rejected from the oral cavity. Both control and chronic decerebrate rats significantly increased their ingestion of sucrose following insulin treatment. Decerebrate rats ingested 2.2 mls of sucrose on saline days and 4.7 mls and 5.5 mls when tested following injection of 5U/kg and 10U/kg, respectively. Control rats had a higher baseline sucrose consumption ($\bar{x}$=9.8 ml) than decerebrates and ingested 13.0 mls and 15.0 mls of sucrose following injections of 5U/kg and 10U/kg, respectively. Insulin injections did not augment ingestion of water over that consumed following saline treatment for either group. Therefore, insulin treatment did not facilitate ingestion nonspecifically. These data indicate that the isolated caudal brainstem is a sufficient neural substrate for the integration of signals generated by insulin and taste factors to control ingestive responses. (Supported by NIH Grant AM 21397 to HJG and NIMH Grant T32-MH15012 to FWF.

76.1 DIRECT-BINDING EVIDENCE FOR MULTIPLE STATES OF S-2 SEROTONIN RECEPTORS. George Battaglia* and Milt Titeler, Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8.

Peroutka and Snyder (1) defined S-1 receptors as serotonin receptors labelled by ^{3}H-serotonin, displaying low affinity for the antagonist spiperone; they defined S-2 receptors as serotonin receptors labelled by ^{3}H-spiperone, displaying low affinity for serotonin. On the other hand, Pedigo et al. (2) reported spiperone displayed high affinity for a component of ^{3}H-serotonin binding. These latter results suggested a site may exist possessing high affinity for both ^{3}H-serotonin and ^{3}H-spiperone.

As shown in Fig. 1 we have found that serotonin and serotonin-like drugs display high and low affinity phases of competition for ^{3}H-spiperone binding to S-2 receptors in cortical homogenates. Serotonin antagonists display monophasic curves in competing for ^{3}H-spiperone binding. Similar agonist-antagonist competition profiles have been demonstrated for neurotransmitter receptors linked to adenylate cyclase systems (3). Further work to be reported has revealed high affinity competition for a component of ^{3}H-serotonin binding by the selective S-2 antagonist ketanserin. These and other data to be reported support our hypothesis that in rat cortex ^{3}H-spiperone labels two states of an S-2 receptor and that ^{3}H-serotonin labels the agonist high affinity state of the S-2 site as well as the S-1 receptor (Fig. 2).

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Fig. 1.

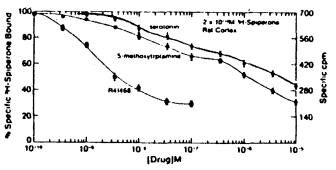
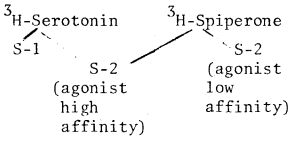


Fig. 2.



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76.2 FACILITATION OF SEROTONIN UPTAKE AND DOWN REGULATION OF IMIPRAMINE RECOGNITION SITES FOLLOWING REPEATED IMIPRAMINE INJECTIONS. M. L. Barbaccia*, N. Brunello, D.-M. Chuang* and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

High affinity binding sites specific for ^{3}H-imipramine have been described and characterized in various brain structures in man and other mammals. A large proportion of these sites are located on serotonergic axons where they appear to be associated with the serotonin uptake mechanism. In various brain structures of rats receiving repeated injections of imipramine (10 mg/kg i.p. twice daily for 3 weeks) the Bmax for the specific binding for this tricyclic antidepressant is down regulated (306±21 fmol/mg protein and 195±18 fmol/mg protein in crude synaptic membranes prepared from hippocampi of saline and imipramine treated rats, respectively). Concomitantly the Vmax of serotonin uptake, measured in slices prepared from hippocampi of imipramine injected rats, is increased (5.4±0.38 ^{3}H-5HT pmol/mg protein/4 minutes and 7.6±0.27 ^{3}H-5HT pmol/mg protein/4 minutes, for saline and imipramine treated animals, respectively).

In brain of rats receiving repeated injections of imipramine there is a down regulation of the Bmax of β-receptor binding sites and a decreased response of the adenylate cyclase to isoproterenol in crude synaptic membranes preparation. These effects depend on the presence of 5HT neurons and appear to be related to the antidepressant action of this drug in man. Hence it can be inferred that repeated imipramine treatments decrease the β-receptor function by decreasing serotonergic synaptic function through a facilitation of the 5HT reuptake. Thus it seems to have an interaction between lowering of 5HT function and down regulation of β-receptors. We propose that an endogenous effector regulates 5HT uptake by acting on the imipramine recognition site and affecting 5HT uptake in an inhibitory manner. When these receptors for this endogenous effector are down regulated uptake increases, 5HT function decreases and β-adrenergic receptor is down regulated. It appears that a system of interneurons connects 5HT receptors to noradrenergic synapses. The location and transmitter characteristics of these interneurons are not known.

76.3 TRYPTOPHAN LOADING INHIBITS SEROTONIN N-ACETYLTRANSFERASE ACTIVITY IN THE RAT PINEAL GLAND. T. S. King, S. Steinlechner*, R. W. Steger*, B. A. Richardson* and R. J. Reiter. Departments of Anatomy and Obstetrics/Gynecology, The University of Texas Health Science Center, San Antonio, TX 78284.

It is generally assumed that the function of pineal serotonin is primarily, if not solely, to act as a substrate for adrenergically-stimulated serotonin N-acetyltransferase (SNAT) activity which rate-limits melatonin biosynthesis. However, the near-absence of serotonin has been shown to augment stimulation of SNAT activity produced by weakly sympathomimetic agents such as pargyline in intact and adrenergically-denervated rat pineal glands (King et al., In: The Pineal and Its Hormones, R. J. Reiter, ed., in press), suggesting that serotonin may exert an inhibiting influence on SNAT activity under certain conditions. Therefore, we sought to determine the effects of tryptophan loading, which is known to increase normally reduced nighttime serotonin levels in the rat pineal gland (Snyder et al., Proc. Natl. Acad. Sci. 53: 301, 1965), on normally high nighttime SNAT activity. Adult male Sprague-Dawley rats were injected with high doses (100 mg/kg) of L-tryptophan at 2400 h (12:12 LD; lights on 0600 h). Two hours later the pineal glands were collected and assayed for SNAT actiyity and catechol-/indoleamine content. Nighttime tryptophan loading led to substantial increases in 5-hydroxytryptophan, serotonin and N-acetylserotonin but a dramatic decrease in SNAT activity in comparison to vehicle controls (0.38 ± 0.13 vs. 5.07 ± 0.70 nmol pineal^{-1} hr^{-1}, respectively). In contrast to other measured amines, melatonin levels were significantly diminished by tryptophan loading. Nocturnally high pineal norepinephrine levels were unaltered by tryptophan loading. The idea that high concentrations of serotonin could lead to substrate inhibition of SNAT activity was not supported by kinetic analysis of vehicle control and tryptophan inhibited SNAT activity under varied substrate concentrations (tryptamine: Km = 1.02 mM; Vmax = 7.48 and 4.76 nmol pineal^{-1} hr^{-1}, respectively; acetyl coenzyme A: Km = 11.52 μM; Vmax = 7.96 and 4.55 nmol pineal^{-1} hr^{-1}, respectively). On the other hand, conjunct treatment with the serotoninergic receptor antagonist cyproheptadine HCl (10 mg/kg) blocked SNAT inhibition by tryptophan loading. This evidence suggests that SNAT activity can be inhibited by high concentrations of serotonin, an apparently postsynaptic effect mediated by serotoninergic receptors. We are currently attempting to define more concisely the role of this proposed serotoninergic receptor mechanism to modulate the well-known adrenergic regulation of pineal SNAT activity. (Supported by Center for Training in Reproductive Biology Postdoctoral Grant HD 07139 and NSF Grant PCM 8003441.)

76.4 EFFECT OF HISTAMINE ON THE ACTIVITY OF SEROTONIN-CONTAINING NEURONS IN THE DORSAL RAPHE. Joan M. Lakoski and George K. Aghajanian, Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Previous studies of the regional distribution of histamine in the CNS have localized high concentrations of this amine in both the hypothalamus and several midbrain nuclei, including the dorsal raphe (DR) (Taylor et al., Brain Res. 41: 171, 1972). While both inhibitory and excitatory effects of microiontophoretically applied histamine have been identified in various brain regions, the physiologcal effects of histamine on DR neuronal activity have not yet been examined. We have, therefore, investigated the effects of histamine applied iontophoretically onto serotonin (5-HT)-containing cells in the dorsal raphe nucleus.

Extracellular single unit activity was recorded in chloral hydrate anesthetized adult male rats; 5-HT cells in the DR were identified by their characteristic slow, regular firing pattern. Iontophoretically applied histamine produced a potent, rapid depression of firing on all 5-HT cells tested (1-5 nA, .05 M in .05 M NaCl). Histamine and GABA were similar in potency, but 5-HT was clearly less potent than histamine in depressing cell firing in the DR. Histamine agonists and antagonists selective for peripheral H1 and H2 receptors were also applied in attempt to characterize the histamine receptors in the DR. Iontophoresis of the H1 antagonists diphenhydramine and mepyramine did not attentuate the histamine response. The H2 antagonists cimetidine and metiamide also did not antgonize the histamine-induced depression of 5-HT cell firing. Rather, both cimetidine and metiamide alone potently depressed cell firing and appeared as more potent agonists than histamine. The H1 agonists 2-methylhistamine and 2-thiazolethylamine and the H2 agonists 4-methylhistamine and impromidine had little or no depressant effects on neuronal activity in the DR.

Iontophoretically applied GABA antagonists picrotoxin and bicuculline rapidly and reversibly antagonized both the histamine and cimetidine-induced inhibition of 5-HT cell firing in the DR. In contrast, the glycine antagonist strychnine did not antagonize this response. The systemic administration of 3-mercaptopropionic acid, an inhibitor of GABA synthesis at doses producing generalized seizures (Karlsson et al., Biochem. Pharm. 23: 3053, 1974), did not alter the depression of DR cell firing by histamine. These data demonstrate that histamine has a potent inhibitory effect on 5-HT cell firing in the DR; although this effect is attenuated by GABA antagonists, it does not appear to be mediated by a facilitation of endogenous GABA. (Supported by USPHS Grants MH-17871, MH-14276 and the State of Connecticut).

76.5 LATERAL HABENULO-DORSAL RAPHE NEURONS CONTROL IN VIVO SEROTONIN RELEASE IN THE BASAL GANGLIA, P. Soubrie, T.D. Reisine*, F. Artaud and J. Glowinski (Spon. H. Reisine). INSERM U. 114, College de France, Paris, France

The importance of the lateral habenula-dorsal raphe pathway in the control of in vivo ^{3}H-serotonin release in the cat basal ganglia was examined using the push-pull cannula technique and an isotopic method for the estimation of ^{3}H-serotonin continuously formed from ^{3}H-tryptophan. ^{3}H-Serotonin was measured in both caudate nuclei and substantia nigra and, in some cases, in the dorsal raphe. Application of potassium to the lateral habenula enhanced ^{3}H-serotonin outflow in both caudate nuclei and substantia nigra. Electrical stimulation of the lateral habenula decreased ^{3}H-serotonin release in all structures studied. Blockade of the GABA inhibitory pathway to the lateral habenula by the local application of picotoxin reduced ^{3}H-serotonin release in both substantia nigra, increased release of the ^{3}H-amine in the dorsal raphe but was without effect on ^{3}H-serotonin release in either caudate nucleus. This inhibition of nigral ^{3}H-serotonin release was antagonized by simultaneous application of picrotoxin into the dorsal raphe. Substance P delivery to the dorsal raphe produced the same effects on ^{3}H-serotonin release as described for picrotoxin application in the lateral habenula except that inhibition of nigral ^{3}H-serotonin release was not prevented by local coadministration of picrotoxin. These results suggest that the lateral habenula can control ^{3}H-serotonin release in the basal ganglia and that this regulation may be different for those serotonergic neurons innervating the caudate nucleus versus those projecting to the substantia nigra.

76.6 THE ACUTE EFFECTS OF AMPHETAMINE AND METHAMPHETAMINE ON THE SEROTONERGIC SYSTEM OF THE RAT BRAIN. M.A. Peat,* G.R. Hanson and J.W. Gibb (SPON: W. Stevens). Dept. of Biochem. Pharmacol. and Tox., University of Utah, Salt Lake City, Utah, 84112.

Three hours after the acute administration of 10 mg/kg of methamphetamine (METH), tryptophan hydroxylase (TPH) activity, serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) concentrations were decreased in both the neostriatum and cerebral cortex. In this study, the effects of varying doses (1.0, 2.5, 10 and 15 mg/kg, i.p.) of METH and amphetamine (AMP) on the neostriatal and cortical serotonergic systems were compared. TPH activity was measured as described previously (Hotchkiss et al., Life Sci., 25, 1373, 1979). 5-HT, 5-HIAA and tryptophan (TRY) concentrations were measured by HPLC-fluorescence. The following data were obtained (expressed as % of saline controls).

	DOSE (mg/kg)	REGION	TPH	5-HT	5-HIAA	TRY
AMP	1.0	Cerebral	113 ± 4	94 ± 4	96 ± 5	107 ± 5
	2.5	Cortex	111 ± 11	92 ± 6	91 ± 5	101 ± 8
	10		75 ± 6*	92 ± 6	86 ± 7	116 ± 10
	15		57 ± 9*	91 ± 5	74 ± 11	136 ± 15
METH	1.0		95 ± 7	91 ± 5	100 ± 3	117 ± 7
	2.5		91 ± 4	109 ± 5	113 ± 5	121 ± 6*
	10		73 ± 5*	53 ± 13*	69 ± 4*	139 ± 5*
	15		35 ± 21*	30 ± 12*	56 ± 10*	165 ± 5*
AMP	1.0	Neostriatum	99 ± 5	101 ± 9	110 ± 3	120 ± 4*
	2.5		79 ± 20	102 ± 9	111 ± 5	111 ± 6
	10		71 ± 4*	108 ± 5	113 ± 9	132 ± 9*
	15		58 ± 14*	104 ± 10	87 ± 13	153 ± 16*
METH	1.0		95 ± 8	99 ± 5	101 ± 7	118 ± 6*
	2.5		89 ± 16	106 ± 6	121 ± 7*	116 ± 4*
	10		55 ± 5*	80 ± 8*	84 ± 2*	123 ± 6*
	15		34 ± 16*	61 ± 15*	69 ± 5*	147 ± 3*

*$p < 0.05$

p-Chloroamphetamine also decreased neostriatal and cortical 5-HT and 5-HIAA concentrations three hours after a single dose (2.5, 10 and 15 mg/kg i.p.). The effects of the drugs were also compared over a 24-hour period following an acute injection (15 mg/kg i.p.). The differences in the effects of AMP and METH may be due to varying distribution of the two agents or to subtle differences in the responses of the serotonergic system to the two amines. (Supported by USPHS Grant DA 00869).

76.7 EVIDENCE FOR HOMOLOGOUS FAMILIES OF DOPAMINE (DA) AND SEROTONIN (5-HT) CONDENSATION PRODUCTS IN CEREBROSPINAL FLUIDS (CSF) FROM MONKEYS. Michael A. Collins, Kristine Dahl*, William P. Nijm* and †Leslie F. Major*. Dept. of Biochemistry, Loyola Univ. Med. School, Maywood, IL 60153 and †Dept. of Psychiatry, SUNY Upstate Med. Center, Binghamton, NY 13901

Chromatographic evidence on multiple liquid chromatography (HPLC) columns and confirmatory capillary gas chromatography (GC) indicates that homologous tetrahydroisoquinoline (TIQ) and tetrahydro-beta-carboline (TBC) condensation products related to or derived from DA and 5-HT are endogenous constituents of primate CSF, and that chronic ethanol (EtOH) exposure may alter the amounts of several of these alkaloids. HPLC/electrochemical screening procedures with a spectrum of TIQ and TBC standards were developed employing both reversed phase (Waters μBondapak) and 10 micron cationic exchange (Toyo Soda Co.) columns. CSFs obtained by cannulation of the 5th lumbar space of Rhesus monkeys before, during and after chronic exposure to EtOH liquid diets, were separated initially into amino acid and amine fractions using a weak cation exchange resin (BioRex 70). The amino acid fractions consistently contained three components which displayed chromatographic identity on the two HPLC systems and upon capillary GC (electron capture detection) assay with the pyruvic acid-derived alkaloids of DA, O-methyl-DA, and 5-HT (1-carboxy-lated TIQs and TBCs). The alkaloid levels were in the mid-(5-50) ng/ml range and, in some monkeys, were increased somewhat by EtOH ingestion. Other 1-carboxyl-TIQs derived from DA and pheylpyruvic acid derivatives were either not detectable or not GC confirmable. Similarly, the amine fractions from 80% of the CSFs contained three constituents which were identical by HPLC and GC with the TIQs and TBCs theoretically derived from acetaldehyde condensation with the aforementioned open-chain amines; Levels were somewhat less than the 1-carboxyl analogs and were not obviously changed by EtOH, but sample limitations prevented definite quantitative conclusions. There is growing but conflicting evidence for the occurrence of "mammalian alkaloids" derived from aldehydes or phenylpyruvic acids (rev: Melchior and Collins, CRC Crit. Rev. Toxicol. 10, 313, 1982). Our results constitute the first experimental indications that homologous families of TIQs and TBCs, related via pyruvic acid and potentially through decarboxylation processes, may be normal biogenic amine derivatives in the nervous system. [Supported by AA00266. Portions of this study were done when L.F.M. was a staff psychiatrist at NIMH]

76.8 CEREBROENDOTHELIAL CULTURES: METABOLISM AND SYNTHESIS OF 5-HT. C. Maruki*, M. Spatz, I. Nagatsu* and J. Bembry*. Lab. of Neuropath. & Neuroanat. Sci., NIH, Bethesda, Md. 20205 and Fujita-Gakuen Univ., Sch. of Med., Toyoake, Aichi 470-11, Japan.

It is a well known fact that 5-hydroxytryptamine's (5-HT) passage across the blood brain-barrier (BBB) is limited under physiological conditions. Our previous studies have shown that 5-HT is taken up by specific temperature, Na^+ and K^+-dependent carrier mediated process in the isolated cerebral microvessels where it is metabolized to 5-hydroxyindole acetic acid (5-HIAA) (Spatz et al., Brain Res., 220, 214-219, 1981). However, this model system does not fully permit the examination of endothelial properties per se since a variable number of vessels (larger than capillaries) composed of endothelium, muscle cells, pericytes and nerve endings are included in such preparation. Therefore, these studies were extended to pure endothelial cultures obtained and propagated from dissociated cells of cerebrovascular fractions.

In this communication we will demonstrate the existence of 5-HT in the endothelium and provide evidence of endothelial ability to metabolize and synthesize 5-HT.

The endothelium used for the investigations consisted of 2nd to 4th generation of cells cultured for 7-10 weeks. The endothelial presence of 5-HT was demonstrated by immunohistochemistry and the content of 5-HT and 5-HIAA was determined in the cultured cells, as well as in medium (consisting of medium 199 and 20% of fetal serum) by high pressure liquid chromatography.

A slight to marked diffuse immunofluorescence specific for 5-HT was seen in the endothelial cultures. The levels of 5-HT and 5-HIAA in the feeding solution obtained from the cultured cells markedly differed from those found in the medium incubated without the cells. The daily monitored concentration of 5-HT decreased, but the level of 5-HIAA remained constant in the former solution, whereas the 5-HT and 5-HIAA content continuously decreased in the latter medium. 5-HT was found in the cultured cells exposed to artificial medium containing L-tryptophan and pargyline, but none was present in sister cultures exposed to balanced salt solution and pargyline.

These findings clearly indicate that the cultured cerebrovascular endothelium was not only able to take up and metabolize 5-HT but also to synthesize this amine from tryptophan. Although the physiological role of the formed 5-HT is uncertain, its vasoactive properties suggest that it might be involved in the regulation of cerebral microcirculation and the BBB integrity.

76.9 REVERSAL OF HYPERSEROTONERGIC ANXIETY WITH GENERATED ANIONS IN HUMAN SUBJECTS. A. James Giannini, Sam Castellani, M.C. Giannini*. Psychiatry Dept., Northeastern Ohio Universities College of Medicine, Rootstown, OH 44222.

Previous studies in this laboratory (Giannini 1979, Giannini and Loiselle 1982) have established a direct correlation between high levels of atmospheric cations, increased levels of serum serotonin and perceived anxiety in human subjects. These were consistent with clinical observations of Sulman et.al.(1972). The study below describes a successfull attempt to use generated anions to treat a hyperserotonergic anxiety state. Seventeen young males volunteered to participate and signed standard consent forms. Each was given a standard laboratory profile and found to be free of pathology of thyroid, pancreatic or adrenal function. They were then collectively exposed continously for ten hours to a cationized atmosphere artifically created by a process described previously (Robinson and Dirnfield 1954). At the end of this period eight subjects reported anxiety. Of this subgroup all demonstrated a resting tremor, two demonstrated flushing and one subject had borborygmi. No other signs associated with a hyperserotonergic state were found in the affected subgroup. The unaffected subgroup presented with no physical findings. The entire group was then exposed for five hours to an anionic field produced by an Amron Ionizer. At the end of this time both subgroups were asymptomatic, Both the previously affected subgroup and unaffected subgroup reported a generalized feeling of well-being. The production of anxiety with a cationic field in 44% of the subjeets is consistent with previous clinical and controlled populations which displayed a sensitivity in 35% to 50% of their patients. The amelioration of these symptoms with anions suggest a nonpharmacological treatment for those occupationally exposed to cationized environments. It also partially replicates the observation of Olivier (1962) who reported relief of stress-induced anxiety with anion aerosols in a rat population.

76.10 PROPHYLACTIC EFFECTS OF AMBIENT ANIONS ON HYPERSEROTONERGIC ANXIETY. Sam Castellani, A. James Giannini, Matthew C. Giannini*. Psychiatry Department, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44222.

In other investigations conducted in this laboratory a correlation has been demonstrated between high levels of ambient cations, increased plasma serotonin and anxiety. The authors have also reversed the anxiety with generated atmospheric anions (1982). In the experiment below a previous exposure to generation anions was studied for possible prophylaxis to cation-induced anxiety. Twelve young males signed standard consent forms to participate in the study. Physical examination and laboratory studies were within normal limits. All were collectively exposed to an anionic field generated by an Amron Ionizer for a ten-hour period. At the end of this time they all reported feelings of well-being. Physical examination results were unchanged. They were then exposed to a cationized field for an additional ten hours. Again there were no subjective nor objective findings. One week later this same group was exposed to a cationized for ten hours. This time eight of the volunteers reported anxiety. And in the affected subgroup there was one report of vertigo. Physical examination revealed a resting tremor in six of the eight affected subjects and flushing in one these six. Laboratory profiles were within the normal range. These observations are consistent with a prophylactic effect of generated anions to anxiety produced by high levels of atmospheric cations.

77.1 ULTRASTRUCTURAL DIFFERENCES BETWEEN SLOW AND FAST NEUROMUSCULAR JUNCTIONS OF MANDUCA. M.B. Rheuben, Department of Anatomy, Michigan State University, East Lansing, Michigan 48824.

Arthropod skeletal muscle fibers can be innervated by excitatory motor neurons that produce either a large amplitude, non-facilitating "fast" excitatory junction potential (e.j.p.) or a small, longer lasting, facilitating "slow" e.j.p. In Manduca, the flight muscles, which themselves may be of tonic, phasic, or intermediate fiber types, can be innervated by various combinations of fast and slow nerves. Using freeze-fracture, scanning, and thin-section techniques, we have compared the structural features of two types of neuromuscular junctions for aspects that might relate to their different performances. The junctions of the middle 3rd axillary muscle (slow nerve, tonic muscle fiber) differ from those of the subalar or dorsal longitudinal muscle (fast nerve, phasic muscle fiber) in several ways. 1) The slow junctions are shorter, (5-20μm, determined from S.E.M. measurements) than the fast (27-37μm) in overall length. 2) Freeze-fractured preparations show that within a slow junction the shape of the nerve terminal is very irregular and varicose compared to that of a fast junction, whose roughly cylindrical terminal is notable for the even alternation of contacts from muscle and glial processes (Rheuben and Reese,1978). 3) Regions of close contact between the nerve terminal and the muscle fiber are oval in shape and appear as "plaques" in both types of nerve. Plaques and active zones on slow terminals occur mainly on the lobes of the varicosities. Plaques on fast terminals are evenly spaced along the sides of the terminal. 4) The plaques themselves are not substantially different in size or shape as observed in freeze-fracture: 0.67μm, average of width and length in slow terminals, and 0.65μm for fast terminals. Thin section measurements of lengths of thickened, electron-dense post synaptic membranes yielded 0.63μm for the slow terminal and 0.73μm for the fast. 5) The particle band associated with the presynaptic active zone is more irregular and somewhat shorter in slow terminals (0.21μm slow, n=51, 0.23μm fast, n=19). The characteristic features of slow n.m.j.'s appear to be a shorter terminal and subsequent shorter total length of active zone, and a more variable diameter of terminal. A smaller e.j.p. would be produced if the length of active zone limits synchronous release. The longer time course could be related to delayed conduction through constricted portions of the terminal. The question of whether these differences are specific to the nerve or whether they are partly dependent upon the type of muscle fiber that is being innervated will not be answered until other combinations of nerve and muscle have been considered.

77.2 THE NUMBER OF SYNAPTIC BOUTONS TERMINATING ON AUTONOMIC NEURONS IS CORRELATED WITH CELL SIZE. Peter B. Sargent, Dept. of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305

The zinc-iodide osmium (ZIO) technique has been used to stain synaptic boutons terminating on parasympathetic neurons in the cardiac ganglion of adult Xenopus laevis. The ZIO technique uniformly and densely stains all synaptic boutons in all preparations (150 synapses examined by electron microscopy in 6 animals). Light microscopic examination of the unipolar ganglion cells in intact tissue reveals that larger neurons have more boutons. The number of boutons terminating on the cell body is strongly correlated with its surface area ($n=200$; $r=0.78$, $p<0.001$); the relationship is approximately linear and passes through the origin. Thus the cell bodies of small neurons have the same density of boutons on their surface as do the cell bodies of large neurons. The size of boutons is similar for small and large cells; thus, on the average, a constant fraction (2%) of the cell body is covered by synapse, regardless of cell size. An expected consequence of this proportionality is to normalize the efficacy of transmission for synapses on cells of varying sizes.

The correlation between bouton number and cell size may arise because boutons "know" of one another's presence on the cell surface; specifically, there may be negative interaction between boutons such that they tend to be evenly spaced. Two types of analyses show that this does not occur. In one, ganglion cells were divided into segments having equal surface areas, and the number of boutons falling on each segment was noted. A comparison of the observed frequency distribution of boutons-per-segment with that expected from the Poisson distribution indicates that boutons are distributed non-randomly: in fact they tend to cluster ($P<0.01$ by the chi-square goodness of fit test). In a second test the distribution of bouton numbers per whole cell was compared to that expected from the Poisson distribution to learn whether boutons are "assigned" to cells independently. The observed frequency distribution of boutons-per-cell was similar to that expected assuming independent trials ($P=0.87$-0.98). Thus the probability that a bouton will be "assigned" to a particular cell is independent of how many other boutons are present on that cell.

The correlation between bouton number and cell size is adequately explained without invoking interaction between boutons. The only factor that influences the probability that a bouton will be assigned to a particular cell is cell size.

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77.3 MORPHOLOGICAL CHANGES IN THE DENTATE MOLECULAR LAYER ACCOMPANYING LONG-TERM POTENTIATION. C. L. Anderson* and E. Fifková (SPON: H. Alpern). Dept. of Psychol., Univ. of Colorado, Boulder, Colorado 80309.

In previous experiments we have shown an increased spine area and perimeter of dendritic spines in the dentate molecular layer following tetanic stimulation of the entorhinal area. Because quick-freezing fixation was used, these experiments could not be electrophysiologically controlled. In present experiments, with an aldehyde fixation, the long-term potentiation could be monitored. Potentiation was elicited with high frequency burst (7 mice) or with non-burst stimulation (30 Hz for 5-30 sec; 13 mice). Controls (10) were stimulated with non-potentiating frequencies (0.2 Hz for 3 min). Both types of stimuli elicited long-term potentiation, however, the burst stimulation was more effective and gave a larger increase of the population EPSP above the baseline. Results of the morphometric measurements (in percentile differences) are summarized in a table below. The spine area and perimeter were enlarged when potentiation was induced with both modes of stimulation; however, the increase was larger in the burst than in the non-burst potentiated group. Likewise, the synaptic length was increased only with burst stimulation which suggests that the mode of stimulation could be a factor determining the type of newly formed proteins. Thus, with two different methods of electron microscope fixation, we have demonstrated in the dentate molecular layer stimulation induced spine enlargement which is likely to be the mechanism of long-term potentiation. The composition of the spine cytoplasma, which could be responsible for the morphometric change, is discussed in the following abstract.

Spine Area	Dentate Molecular Layer		
Mode of Stimulation	P/3	M/3	D/3
BP-NP	3.1 ± 5.2	20.0 ± 5.5	20.9 ± 4.4
p	NS	<0.01	<0.01
NBP-NP	3.0 ± 4.1	12.3 ± 4.3	12.0 ± 3.6
p	NS	<0.05	<0.01
Spine Perimeter			
BP-NP	-5.1 ± 2.7	8.4 ± 3.1	8.5 ± 2.1
p	NS	<0.05	<0.01
NBP-NP	3.4 ± 2.4	9.1 ± 2.1	5.7 ± 2.1
p	NS	<0.01	<0.05
Synaptic Length			
BP-NP	12.0 ± 5.9	22.8 ± 5.2	21.8 ± 4.8
p	NS	<0.01	<0.01
NBP-NP	0.7 ± 6.4	2.5 ± 5.0	8.7 ± 5.6
p	NS	NS	NS

Groups: BP=burst potentiated; NBP=non-burst potentiated; NP=non-potentiated. (Supported by MH 27240-07.)

77.4 CYTOPLASMIC ACTIN IN DENDRITIC SPINES AS A POSSIBLE MEDIATOR OF SYNAPTIC PLASTICITY. E. Fifková and R. J. Delay.* Dept. of Psychology, Univ. of Colorado, Boulder, Colorado 80309.

Following permeation with saponin and incubation with S-1 fragment of myosin we have demonstrated at the ultrastructural level that the cytoplasmic actin is organized in long filaments in dendritic spines, dendrites, axons and axon terminals in the dentate molecular layer. The actin filaments, interwoven in a network, fill entirely the spine head. In the spine stalk they appear more dense than in the spine head and are arranged in braided strands similar to those associated with microtubules in dendrites and axons. In the high voltage EM, the spine apparatus appears to be suspended in the actin filament network rather than associated with it. The filaments are clearly associated with the plasma membrane and with the postsynaptic density (PSD) vertically and also in parallel. Both types of actin filament association with the spine membrane together with the organization of actin filaments within the spine may be important in controlling the shape and dimensions of the spine. Since nonmuscle actin binds reversibly to myosin, it is likely that in nonmuscle cells, including neurons, the actin-myosin interaction may be regulated similarly to the muscle. It has been shown that myosin, labeled with fluorescent antibodies, has the same location in the cell as actin, yet with the EM thick myosin fibers were not demonstrated. Therefore, it has been speculated that the cytoplasmic actin may have a support-providing rather than a contractile function. However, given that only a few myosin molecules are needed to generate the small force involved in nonmuscle contractibility, then even in the absence of myosin fibers, the myosin molecules could be provided by the cytoplasmic ground substance. Furthermore, actin filaments of an in vitro gelated cytoplasma contract in the presence of Ca^{2+}. This inherent contractibility of actin filaments, their high concentration in the spine head and their strand-type organization in the spine stalk suggest that under certain conditions of excitation the length and width of the spine stalk and thus the electrical properties of the spine could change as was predicted by Rall (1974). The necessary Ca^{2+} could be provided either by an inward Ca current or by intraneuronal Ca sources. Thus, actin could be implicated in widening and shortening of the spine stalk and in enlargement of the spine head, i.e., in morphological changes which occur concurrently with long-term potentiation and which were postulated to be the mechanism of the enhanced synaptic strength. Similar changes in the cytoskeleton could to a lesser extent occur also during physiological activity per se. Furthermore, the ubiquitous presence of actin in all the neuronal processes suggests that it could be the common denominator of synaptic placticity in general. Supported by MH 27240-07 and EY 01500-07.

77.5 SYNAPTIC VESICLES AND TRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION. C.K. Meshul and G.D. Pappas. Dept. of Anatomy, Univ. of Illinois Med. Ctr., Chicago, Illinois 60612

It has been generally assumed that transmitter in synaptic vesicles is released by exocytosis and that new vesicles are reformed by the recovery of presynaptic membrane by endocytosis. Coated vesicles may be first formed and then become transformed to the usual uncoated synaptic vesicles. The aim of the present study was to clarify the fate of synaptic vesicles following indirect electrical stimulation or K^+-depolarization. After intense transmitter release and a subsequent rest period, we determined whether synaptic vesicles recycle as coated vesicles and to what extent the synaptic and coated vesicles become labelled with the extracellular tracer, horseradish peroxidase(HRP). Also we studied whether the extent of labelling correlated with the number of vesicles released. The sartorius muscle of the frog, incubated in vitro with frog saline containing HRP, was either indirectly stimulated at 10 Hz or K^+-depolarized for 15 minutes, then subsequently rested for 15 minutes. Identified muscle fibers were monitored electrophysiologically and their respective nerve endings later identified under the electron microscope. There was a statistically significant decrease in the mean number of vesicles/section following electrical stimulation, but not after K^+-depolarization. No difference was found in the mean number of vesicles/section following the 15 minute rest period. This was despite the fact that the amplitude of the end plate potential was about 25% of the control. It was also found that less than 10% of the mean number of vesicles/section were labelled with HRP. The number of coated vesicles increased following electrical stimulation or K^+-depolarization, but their numbers accounted for less than 2% of the mean number of vesicles/section. It was also shown that a surprisingly high number of coated vesicles contained no HRP. Experiments were also done to rule out any deleterious effects of HRP on the number of vesicles remaining after electrical stimulation or K^+-depolarization. There was no statistical difference in the mean number of synaptic or coated vesicles/section in HRP versus non-HRP experiments. It is concluded that these findings are inconsistant with recycling of synaptic vesicle membrane exclusively via coated vesicles and the exclusive role of synaptic vesicles in transmitter release. The data reported in this study is consistant with the hypothesis that distinguishes two pools of transmitter within the nerve terminal. It is suggested that cytoplasmic acetylcholine may be the source of transmitter for evoked release, and vesicular acetylcholine the source for spontaneous release.

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77.6 ORGANIZATION OF FILAMENTS IN PRESYNAPTIC AND POSTSYNAPTIC CYTOPLASM REVEALED IN RAPIDLY FROZEN CEREBELLAR CORTEX. D.M.D. Landis and T.S. Reese. Dept. Neurology, Massachusetts General Hospital, Boston, MA; LNNS, NINCDS, NIH, Bethesda, MD. 20205

Spines are a common aspect of neuronal architecture, but their function and the mechanisms which maintain their shape are unknown. We examined cytoplasmic and membrane structure associated with synaptic junctions on Purkinje cell dendritic spines, and have found several distinct filament systems.

Mouse cerebellar cortex was rapidly frozen by contact against a copper block cooled by liquid helium and fractured at 10-85°K on the stage of a modified Balzers freeze-fracture apparatus, helium-cryopumped to a vacuum of 10^{-8} torr. When the tissue was etched prior to replication, sublimation of water from the frozen surface of the tissue revealed three distinct sets of filaments in dendritic spines of Purkinje cells. A skein of filaments, each 5-7nm in diameter without obvious periodicity, filled the bulb of the spine and extended root-like into the parent dendritic shaft. These filaments ended in apposition to the true inner surface of the spine membrane, except at the synaptic junction. A second set of shorter, 4-6nm diameter, branched filaments extended 20-50nm into the spine cytoplasm from a zone on the true inner surface of the spine membrane which was coextensive with the widened synaptic cleft. These filaments formed a mesh which probably corresponds to the electron-dense fuzz seen in aldehyde-fixed, thin-sectioned tissue. Finally, a third set of sparse, actin-like filaments, 9-10nm in diameter with a subtle 4nm periodicity, was present deep to the synaptic junction and in the core of the spine. No filaments were within the lumen of membrane cisterns in the spine.

In the axoplasm of the parallel fiber boutons presynaptic to spines, somewhat pleomorphic filaments 5-7nm in diameter were interlaced among synaptic vesicles clustered near the junction. That region of presynaptaic plasmalemma which is indented by the widened synaptic cleft was contacted by similar filaments which extended from the true inner surface to surround nearby synaptic vesicles. Away from the site of the widened synaptic cleft, filaments did not extend from vesicles to the axon membrane. Each of these filament systems in pre- and post-synaptic processes is visible in rapidly-frozen tissue prepared for thin-section study by freeze-substitution fixation.

We suggest that the 5-7nm and 9-10nm actin-like filament systems in Purkinje cell spines may constrain their shape, and may represent a mechanism for modulating spine shape. Filaments in the axoplasm may interact with synaptic vesicles and move them toward the site of fusion with the presynaptic membrane. (Supported in part by NS 15573 and NS 00353).

77.7 THE PUTATIVE 51,000 M_r PROTEIN MARKER FOR POSTSYNAPTIC DENSITIES IS VIRTUALLY ABSENT IN CEREBELLUM. Steven D. Flanagan, Beverly Yost* and Garrett Crawford*. Division of Neurosciences, City of Hope Research Institute, Duarte, California 91010.

Cerebrum and cerebellum contain numerous asymmetric synapses characterized by the presence of a postsynaptic thickening prominently stained by phosphotungstic acid and other electron dense stains suitable for electron microscopy. A 51,000 M_r protein, copurified in postsynaptic density enriched fractions from cerebrum, is considered to be a well established marker for the postsynaptic density. Based upon two criteria, our studies demonstrate that the 51,000 M_r protein marker for postsynaptic densities is virtually absent in cerebellum. First, based upon electrophoresis and subsequent peptide mapping analysis, it is present in negligible amounts in deoxycholate insoluble fractions from cerebellum, but abundant in parallel fractions from cerebrum. Secondly, the 51,000 M_r protein, which binds ^{125}I-calmodulin after $NaDodSO_4$/polyacrylamide gel electrophoresis, is readily visualized in membranes samples from cerebrum but is present at levels twenty-fold lower in the parallel cerebellar synaptic plasma membrane fraction.

This confusing situation could be remedied by assuming that the mPSDp is an authenic marker for Type I PSD structures present in the cerebrum, but rare in the cerebellum. If the mPSDp is, indeed, a component of cerebral Type I synapses, then the fact that the mPSDp is virtually absent in cerebellum implies that cerebellar and cerebral Type I synapses must now be further delineated, both structurally and in terms of the role of calmodulin in synaptic activity. However, the evidence that the mPSDp is an authenic component of the PSD is based entirely upon the results of subcellular distribution studies, by correlation of ultrastructural and electrophoretic analysis.

A further, but preliminary, observation may bear upon the 51,000 M_r protein's role in synaptic structures. We observe that a soluble cerebral ^{125}I-calmodulin binding protein co-migrates electrophoretically with the cerebral 51,000 M_r deoxycholate insoluble protein. Peptide mapping analysis, currently underway, may serve to determine whether this protein is homologous to the deoxycholate insoluble species. Since the 51,000 M_r protein is known to be a prominent substrate for calmodulin stimulated protein kinase activities, this observation prompts the working hypothesis that soluble 51,000 M_r calmodulin binding protein could represent a phosphorylated or unphosphorylated form of the 51,000 M_r species.

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77.8 PURIFICATION AND CHARACTERIZATION OF A Ca^{++}-CALMODULIN DEPENDENT TUBULIN KINASE FROM RAT BRAIN CYTOSOL. J.R. Goldenring, B. Gonzalez* and R.J. DeLorenzo. Dept. of Neurology, Yale Medical School, New Haven, CT 06510

Calcium and calmodulin have been implicated in the regulation of many cellular functions, and their interaction with tubulin may play a role in many dynamic processes in neurons (Fed. Proc. 41: 2265, 1982; Cell Calcium 2:365, 1981; Ann. N.Y. Acad. Sci. 356:92, 1980). We have previously demonstrated the existence of Ca^{++}-calmodulin dependent tubulin kinase activity associated with synaptosomes, synaptic cytoplasm and synaptic vesicles (PNAS 78:991, 1981; Brain Res. 236:393, 1982; J. Neurochem. 38:1205, 1982). We now report the purification and characterization of a tubulin kinase from rat brain cytosol.

In an attempt to separate the kinase activity from the acidic endogenous proteins tubulin and calmodulin, a chelated high speed supernatant from rat brain was subjected to cellulose phosphate chromatography and eluted with a NaCl gradient. Ca^{++}-calmodulin dependent exogenous tubulin phosphorylating activity was eluted from the resin as a peak with 250 mM to 350 mM NaCl. This fraction was free of significant amounts of either calmodulin or tubulin as assessed by 2-dimensional gel electrophoresis. This fraction was then chromatographed on calmodulin-affinity resin in the presence of 2 mM $CaCl_2$. The column was washed sequentially with 50 mM NaCl, 200 mM NaCl, and finally 200 mM NaCl with 2 mM EGTA and 2 mM EDTA. Tubulin kinase activity eluted as a peak with chelator wash. We have previously reported that brain cytosol contains a tubulin filamentous polymerizing activity (Brain Res. 236:393, 1982). This in vitro Ca^{++}-calmodulin dependent polymerizing activity copurified with the kinase activity.

Both cellulose phosphate and calmodulin-affinity purified calmodulin kinase fractions contained a single major calmodulin binding protein of approximately 50,000 daltons, as assessed by ^{125}I-calmodulin overlay of PAGE gels. Thus, this protein is likely to be either the kinase itself or a subunit of the kinase. In addition, synaptic membrane was also shown to contain a calmodulin binding protein of the same molecular weight. This suggests that either the same kinase may exist in both membrane bound and unbound forms, or different kinases may have, at least, the same 50,000 daltons calmodulin binding subunit in common.

The structure of the kinase and its relation to the in vitro filamentous polymerization of tubulin will be discussed.

77.9 Ca^{2+}-CALMODULIN KINASE DEPENDENT FILAMENTOUS POLYMERIZATION OF TUBULIN. R.J. DeLorenzo, J.P. Albert*, and P.R. DeLucia* (SPON: Gilbert H. Glaser). Dept. of Neurology, Yale Medical School, New Haven, CT 06510

Previous studies from this laboratory (PNAS 78: 991, 1981; Brain Res. 236: 393, 1982; J. Neurochem. 38: 1205, 1982) have characterized an endogenous Ca^{2+}-calmodulin tubulin kinase system in brain cytosol, synaptoplasm, synaptic membrane, and synaptic vesicle preparations. It has been suggested from these results that the Ca^{2+}-calmodulin tubulin kinase system may mediate some of the effects of Ca^{2+} on cellular activity and synaptic function (Fed. Proc. 41: 2265, 1982). We now report that the phosphorylation of tubulin by the Ca^{2+}-calmodulin kinase system results in a marked physiochemical alteration in the properties of tubulin, resulting in the non-random formation of filamentous tubulin polymers that are clearly distinct from microtubules. It was observed that conditions producing phosphorylation of tubulin in cytosol, synaptoplasm, and synaptic vesicle preparations caused a significant turbidity change in the reaction mixture. Isolation by centrifugation of the aggregated materials produced by Ca^{2+}-calmodulin dependent protein phosphorylation in each fraction revealed that phosphorylated tubulin was the major protein component in the pellet. To clearly correlate tubulin phosphorylation with the formation of tubulin aggregates, we isolated a tubulin kinase preparation that was free of endogenous tubulin and calmodulin. This tubulin kinase fraction was shown to phosphorylate highly purified tubulin in a Ca^{2+}-calmodulin dependent fashion. Employing this system it was shown that phosphorylation of tubulin by the kinase preparation caused a rapid aggregation of tubulin into filamentous polymers. These tubulin aggregates were not solubilized by treatment with high salt, EGTA, EDTA, or cold temperatures. Removal of tubulin phosphate by base hydrolysis or phosphatase activity solubilized the tubulin structures. Electron-microscopic studies of negatively stained, rotary shadowed, thin sectioned, and freeze fractured preparations revealed that phosphorylated tubulin was aggregating in an ordered fashion with the production of linear polymers of 10-12 nM diameter that twisted into thicker filaments of 21-23 and 41-42 nM diameters. Affinity purified tubulin antibodies absorbed to colloidal gold specifically labelled the tubulin filaments. The tubulin kinase system provides a unique biochemical mechanism for converting the Ca^{2+}-signal into a physiochemical change in tubulin, resulting in the formation of tubulin polymers. The Ca^{2+}-calmodulin regulated phosphorylation of tubulin in nerve cells and in the presynaptic nerve terminal may mediate some of calcium's effects on synaptic function and delineate a dynamic role for tubulin in synaptic events and information processing in the mammalian brain.

77.10 PARTIAL PURIFICATION, CHARACTERIZATION, AND COMPARISON OF SOLUBLE AND PARTICULATE CALMODULIN-DEPENDENT SYNAPSIN I KINASE ACTIVITIES. M. B. Kennedy, T. McGuinness*, and P. Greengard. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125, and Department of Pharmacology, Yale Medical School, New Haven, CT 06510.

Synapsin I (formerly called protein I), a synaptic vesicle-associated neuronal protein, is phosphorylated by cAMP-dependent protein kinase (Ueda and Greengard, 1977, J.B.C. 252:5155), and by two calcium-dependent protein kinases (Kennedy and Greengard, 1981, P.N.A.S. 78: 1293). One of the two calcium-dependent kinases, which is roughly evenly distributed between soluble and particulate fractions of brain homogenates has been purified approximately 100-fold from each fraction. The properties of the two partially purified enzymes have been compared and by all criteria thus far examined, the two are indistinguishable. For example: both are activated by the same physiological concentrations of calcium ion; the activation is mediated by the calcium-binding protein, calmodulin; their protein substrate specificities and affinities for synapsin I and ATP are identical; and they have the same mobilities during DEAE-cellulose chromatography. The conditions that solubilize the particulate enzyme, dilution and low ionic strength, indicate that it is not a tightly-bound membrane protein.

Schulman and Greengard (1978, Nature 271:478) in their initial description of endogenous calmodulin-dependent protein kinase in crude brain particulate fraction, showed that phosphorylation of several particulate proteins was stimulated by calcium and calmodulin. In the course of experiments to test whether the calmodulin-dependent synapsin I kinase could phosphorylate any of these particulate substrates, we made the observation that one of the most prominent substrates, a 50 kdalton (kd) protein, and also a pair of substrate proteins in the 60 kd range, are present in the partially purified enzyme preparations from both the soluble and particulate fractions. The three substrate proteins in the partially purified synapsin I kinase appear identical to the corresponding substrates in the crude particulate fraction when compared by proteolytic phosphopeptide mapping, and by 2-dimensional gel electrophoresis. These proteins can be separated from α and β-tubulin on SDS gels. The 50 kd substrate is the major protein in the partially purified kinase preparations as determined by Coomassie blue staining of SDS and 2-dimensional polyacrylamide gels. The peaks of enzyme activity and of the 50 kd protein coincide during DEAE-cellulose and calmodulin-sepharose chromatography. These results suggest that the 50 kd substrate protein may be an auto-phosphorylated subunit of the synapsin I kinase. Studies on the possible relationship of the 60 kd proteins to the synapsin I kinase are in progress. Supported by MH-17387, NS-08440 (to P. G.), NS-17660-02 (to M.K.), and GM-07205 (T.M.).

78.1 MOTOR CONTROL OF MULTI-MOVEMENT BEHAVIORS: OROFACIAL MUSCLE RESPONSES TO LOAD PERTURBATIONS OF THE LIPS DURING SPEECH. J.H. Abbs* & V.L. Gracco*. (SPON: J. HIND). Speech Motor Control Labs., Waisman Center, University of Wisconsin, Madison 53706

Most investigations of motor control utilizing load perturbations have focused on single joint movements. Often these studies have led to the conclusion that the ascending afferent pathways offer only moderate compensatory capabilities and perhaps this feedback control is functional primarily for correction of very small errors. Seldom, however, has the perturbation paradigm been applied to more natural motor behaviors where several concurrent movements are required. One such motor act, involving multiple, coordinated movements of the orofacial, laryngeal and respiratory systems, is human speech.

In the present studies, inferiorly-directed loads were applied to the lower lip with a DC brushless torque motor operating under force feedback. This instrumentation permitted coupling of the motor to the lower lip with a constant tracking force of 3-5 gms. via a low-friction pivot arm. The loads were applied prior to & during the agonist muscle activity associated with coordinated lower lip-upper lip movements for a "b" sound. Load magnitude was well within the force range of the lower lip musculature (10-40 gms) to enhance interpretation of the responses in relation to 'normal' control mechanisms. Additionally, loads were applied on less than 15% of the movement repetitions to minimize perturbation-induced adaptation or anticipation.

Load-related responses were observed in both the lower & upper lips, indicating the operation of closed loop, feedback & open loop, feedforward control, respectively. These compensations were apparent the first time a load was introduced. Moreover, the speech gesture was never disrupted by the perturbation.

Analyses of response magnitudes as a function of load onset time (re: the initiation of agonist EMG) revealed that the labial closing gesture was always under afferent dependent control at those points where the perturbation would have otherwise caused a disruption. Parallel analyses indicated that the magnitude of the net compensatory response (lower plus upper lip muscles) was sensitive to the full range of load-induced displacement errors (1-6 mm). Finally, it was apparent that feedback and feedforward control processes were operating differentially. In particular, the relative magnitudes of feedforward vs. feedback compensation changed with (1) the timing of the load re: the agonist muscle activity & (2) the velocity and displacement parameters of the induced error. The implications of these findings for investigations restricted to single joint movements and for control of other linked, multi-movement behaviors will be discussed.

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78.2 TEMPORAL RESPONSE CHARACTERISTICS OF THE PERIORAL SYSTEM TO LOAD PERTURBATIONS. V.L. GRACCO* and J.H. ABBS*. (SPON: J. Gibson) Speech Motor Control Labs., Waisman Center, Univ. of Wisconsin, Madison, WI 53706.

The perioral system, consisting of the upper and lower lips and 4-6 associated muscles, provides a unique and complex macrostructure to investigate neural mechanisms underlying control of a skilled motor act. In contrast to the limbs, spindles and tendon organs are absent. Additionally, the perioral brain stem reflex is polysynaptic and sensory innervation is provided by cutaneous mechanoreceptors.

The present investigation is an attempt to discern the potential pathways underlying load compensation in the perioral system via analyses of the latency and morphology of the EMG responses. Responses were obtained from multiple muscles which acted synergistically to close the lips for an "aba" gesture. Inferiorly directed loads were applied to the lower lip at different times prior to and during the agonist EMG burst for lower lip elevation. Load magnitudes of 35-40 gms with a 12 msec rise were applied randomly on approximately 10% of the trials.

Regarding brain stem pathways, there was a notable absence of these short-latency EMG responses during intervals where ensemble averaging could be employed, i.e. prior to and following the agonist burst. Longer-than-brain-stem responses were found to vary in latency as a function of the load onset time re: agonist burst initiation. For loads introduced approximately 75-30 msec pre-EMG onset, latencies ranged from 55-80 msec. Loads introduced 30 msec or less prior to EMG onset resulted in latencies from 26-55 msec. Response morphology was also seen to vary with load onset; as loads were applied later into the agonist burst, a second burst of EMG activity from all synergistic muscles was seen with a latency of 90-115 msec. Finally, response latencies varied for different synergistic muscles. That is, upper lip muscles often exhibited shorter latencies than lower lip muscles possibly reflecting differential sensitivity to certain stimulus parameters (i.e., displacement vs velocity or velocity vs acceleration).

These response latency data appear to argue against the operation of fixed, reflex-like mechanisms in motor behaviors such as speech involving multiple movements and multiple synergistic muscles. Rather, one might posit more flexible, supra-bulbar processes that dynamically change with the temporal/spatial requirements for specific motor behaviors.

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78.3 REACTION TIMES IN HUMAN ARM TRAJECTORY FORMATION. M. Mathison* and W. Abend* (SPON: E. Bizzi). Dept. of Psychol., M.I.T., Cambridge, MA 02139.

Insights into the means by which the nervous system coordinates the large number of degrees of freedom of movement of the human arm can be obtained by investigating the hand trajectories produced during multi-joint arm movements. When performing planar horizontal arm movements, subjects usually move their hands to new locations by way of roughly straight paths (Morasso, P., Exp. Brain Res. 42, 223, 1981). When subjects are explicitly instructed to follow a smoothly curved path to a new hand location, the paths have a segmented appearance, as if the subjects were trying to approximate a curve with a series of low curvature elements. Also, the hand slows at regions where two straight segments meet (Abend et al., Brain, 1982, in press). It is possible that these discontinuities reflect constraints imposed by the arm controller when curved movements are planned or implemented. We have investigated whether there are differences in the production of straight and curved movements by comparing the reaction times (RTs) to initiate the two types of trajectories. A transducer was used to track the hand position during planar horizontal arm movements to targets. Nonconstraining straight and constant-curvature guide paths, which the subject followed with his hand, were presented by a slide projector fitted with a high-speed shutter. An algorithm was used to determine the RT (time from guide presentation to the foot of the hand-speed profile). In 18 of 19 subjects who participated in a simple RT paradigm, the mean subject RT for curved movements was significantly greater than that for straight movements (difference between means for curved and straight movements, range 13-52 msec). A similar finding was obtained with a choice RT paradigm and when the RT to EMG onset was measured. The results of a variety of control experiments suggest that this finding is independent of perceptual, work space, joint rotation, movement length and movement duration factors.

The results suggest that the intended hand-trajectory characteristics may influence the RT of a movement. If the segmented appearance of curved movements reflects a piecewise trajectory composition, the prolonged RT may reflect the time required to plan or execute multiple segments. If the discontinuities reflect a movement strategy designed to optimize some movement variable, then the increased RT may reflect the optimization procedure. In either case, an influence on the RT by the trajectory characteristics is consistent with the notions that multi-joint arm movements are organized in terms of the hand trajectory and that straight hand paths comprise fundamental movement elements.

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78.4 EVIDENCE FOR AN OPTIMIZATION STRATEGY IN ARM TRAJECTORY FORMATION. T. Flash* and N. Hogan* (SPON: W. Richards). Dept. Psychol., M.I.T., Cambridge, MA 02139.

Studies of planar horizontal arm movements with two degrees of freedom performed by normal subjects instructed to move the hand from one target to another, have shown that the path taken by the hand was usually straight or only gently curved (Morasso, P., Exp. Brain Res. 42, 223, 1981; Abend et al., Brain, 1982, in press). It was found that the speed of the hand was always roughly bell-shaped, whereas joint position and joint velocity varied widely for movements made in different parts of the work space. The independence of the hand-speed profile from the work space is consistant with the idea that the central nervous system plans a movement in terms of hand kinematics. When subjects were instructed to approach targets by way of curved paths, the paths appeared to be composed of a series of gently curved segments. The hand-speed profiles were irregular; valleys of inflection in hand speed were temporally associated with peaks in path curvature. To account for these features, a model based on minimization of the rate of change of hand acceleration (jerk) was tested. Hand trajectories were simulated and compared to experimental records of human planar arm movements. For simulation, the input parameters were the coordinates of the initial and the final hand position, the movement duration, and a via point which corresponded to the maximal curvature point in the recorded trajectories (no via point was required for target to target movements). The fit of simulated to real trajectories was very good for both straight and curved movements regardless of the speed or the region in work space. These results show that a mathematical model based on the rate of change of hand acceleration adequately describes observed hand trajectories. This optimization procedure may be indicative of a central computational algorithm which uses spatial positional cues to generate trajectory plans.

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78.5 FINAL POSITION CONTROL IN SIMULATED PLANAR ARM MOVEMENTS. Jonathan Delatizky. Artificial Intelligence Lab and Dept. of Electrical Engineering & Computer Science, MIT, Cambridge MA 02139

If the motor control system could generate movements by specifying the target configuration of the limb and relying on its intrinsic mechanical properties to shape the resultant trajectory, the difficult problem of path planning and implementation could be much simplified. Since these mechanical properties are complex, it is not clear a priori whether schemes of this type are able to reproduce observed behavior. We have investigated this question by testing a "Final Position Control" hypothesis in two degree of freedom simulated movements. The comparable human movements are reaching movements in the horizontal plane, characterized by almost straight-line hand paths with symmetrical bell-shaped velocity profiles (Abend et al, Brain: in press).

The arm model is kinematically equivalent to a human arm free to move in the horizontal plane with the shoulder girdle restrained. Six muscles are incorporated to provide single- and double-joint flexion and extension at shoulder and elbow. The muscles are modeled as variable stiffness springs with viscosity. First order dynamics are included to model the rise time of muscle force.

The hypothesis tested states that movements are initiated by abruptly changing the innervation of each muscle to that required to establish a static equilibrium at the target position (hence "Final Position Control"). The time course and shape of the resulting movement are entirely determined by the mechanical properties of the arm and muscle models. Since the six muscles form a redundant set for specifying equilibrium, there is considerable freedom to choose muscle commands that satisfy the equilibrium condition. Consequently several methods for determining commands to the individual muscles have been tested.

In all cases movements that terminate at the desired target are generated. The shape of the trajectories usually displays more curvature than do the comparable human movements, while the hand velocity profiles are asymmetrical with an early peak and an apparently overdamped decay. Thus the hypothesized scheme is not by itself an adequate explanation for the observed behavior. However the characteristics of the simulated trajectories suggest that the additional control necessary to realize real movements may in fact be quite simple.

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78.6 PRELOAD EFFECTS ON COMPENSATORY RESPONSES TO LIMB DISTURBANCES. K.M. Newell,* S.L. Marcus* and J.C. Houk. Rehabilitation Institute of Chicago and Department of Physiology, Northwestern University Medical School, Chicago, IL 60611.

This study examined the effects of preload on latency of compensatory responses to flexion-extension perturbations of the wrist in the horizontal plane. In Experiments 1 and 2 the subjects were required to establish an initial flexion or extension force (preload) of 24 N at a prescribed initial muscle length. The perturbations changed the load force by + 8 N in both simple and choice reaction protocols. Latencies of reactions were taken as the time of departure of the compensate force trace from that for a noncompensate trial under the same preload and perturbation. The results showed that the latencies to compensate for the perturbation were longer (by approximately 25 msec) when the perturbation unloaded the muscle. In Experiment 3, reactions in response to a visual stimulus generated no directional effect in relation to these same preloads. In Experiment 4, preload was varied from 0 to 36 N with the same load and unload stimuli as employed in Experiments 1 and 2. The loaded stimuli only produced shorter latencies at the higher preload conditions (24 and 36N). Collectively, these experiments confirm that the latency advantage due to loading is the result of an interaction of the disturbance stimulus and the preload condition.

78.7 SOME FACTORS UNDERLYING SPECIFIC FORELIMB TRAJECTORIES. V.E. Amassian, L. Eberle* and D. Batson*. Dept. of Physiology, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203.

We have studied the quantitative relationships between shoulder, elbow and lumped wrist-digit joint angles during the initial, approximately linear vertical trajectory of many cm during contact placing(CP). Joint angles were computed from the positions of fluorescent shoulder, wrist and digit skin markers, which were illuminated by UV light and recorded with a TV camera. The error due to skin slippage at the elbow was avoided by computing this position trigonometrically, given the distances from elbow to shoulder and to wrist.

The movement vector associated with elbow flexion has both vertical and horizontal components; theoretically, in a set of trials, the horizontal component might be corrected by a particular, i.e. invariant, combination of posterior flexion at the shoulder and ventroflexion at the wrist. However, during the contactual trajectory, the changes in shoulder and wrist angles ($180-\theta_{sh}$, $180-\theta_{wr}$) covaried inversely and were usually related approximately linearly to elbow angle ($180-\theta_{el}$) (Amassian, Eberle and Rudell, J. Physiol. (1981) 310, 51-52P).

These data are fitted by: $d\theta_{sh}/d\theta_{el} = a\ (d\theta_{wr}/d\theta_{el})+b$...eq(1) In 13 cats or kittens displaying economical CP, values of 'a' and 'b' were -0.35±0.07 and 0.78±0.10, respectively. During performance of similar vertical trajectories, $d\theta/dt$ at each joint may vary markedly. Similarly, the integrated EMG varies in a major agonist-biceps. In a given trial, $d\theta/dt$ varies much more than the ratios of the angular velocities ($d\theta_{sh}/d\theta_{el}$, $d\theta_{wr}/d\theta_{el}$), which are either constant or shift (inversely), usually abruptly to new values, which satisfy equation (1). Thus, ratios of angular velocities, related to muscle lengths of synergists acting at different forelimb joints, are the most important variables regulated during the vertical trajectory studied. A reciprocal relationship between synergists acting at shoulder and wrist is crucial in preserving the correct trajectory of the extremity.

Although CP is initiated by cutaneous (largely hair) afferent input which is sustained during a vertical contactual trajectory, such input from the paw could not regulate particular ratios of angular velocities at proximal limb joints. Joint afferent input was curtailed by injecting various forelimb joints (including elbow) with 0.5-1.0 ml of 2% lidocaine and 1:100,000 epinephrine under light ethyl chloride anesthesia (cf Ferrell, J. Physiol. (1980), 299, 85-99). After recovery from anesthesia, CP temporarily became grossly hypermetric after the paw had reached the top of its contactual ascent. However, linear relations between joint angles during contactual ascent were not abolished, suggesting that they depended on spindle afferents.

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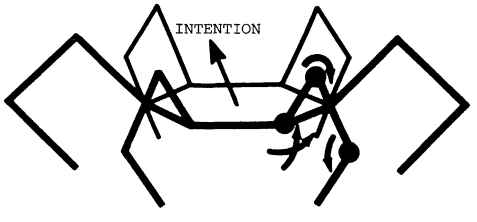
78.8 TENSOR NETWORK THEORY PROVIDING A PARADIGM FOR MOTOR CONTROL OF POSTURE AND MOVEMENT IN MULTILEGGED SYSTEMS. R. Malinow*, A. Pellionisz and R. Llinas. Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave., NY 10016.

A tensorial approach to the function of neural systems has been proposed in recent years (Pellionisz & Llinas, Neuroscience 4:323, 1979,). In this work we further elaborate this paradigm for the control of a crab-like system in 3-space; a six-legged hexagonal platform. The central problem addressed concerns the conversion of a movement intention given, e.g. as a single 3-vector, into a motor execution by a vastly overcomplete motor system with 24 degrees of freedom.



Commands (intentions) are directed towards the change in position of the platform. Displacement of the platform is executed via the angular displacements of the four angles in each limb. Thus an intention specified in a particular coordinate system (e.g. the "normal" Cartesian 3-space coordinates) must be converted into information specifying how much each angle at each joint must move in order to execute this intention properly.

This is accomplished using an embedding of the Cartesian 3-vector covariantly in the natural oblique coordinate system with 24 components, and then expressing the movement contravariantly, with the use of a metric tensor. These transformations have been incorporated into a computer simulation which given intentional and proprioceptive information can display, using computer graphics, a proper execution. A film will be presented to illustrate the results.

The constructive method employed is general (coordinate system free) and may be useful in analyzing biological intention-execution paradigms where the sensory and motor systems may use different natural coordinate systems. Furthermore, this method makes certain predictions as to the nature of the execution of a given intention and thus is amenable to experimental verification. Supported by USPHS grant NS13742.

78.9 MECHANICS OF STANCE AND LOCOMOTION IN PHYSIOLOGICAL COORDINATES: A BIOMECHANICAL MODEL TAKING INTO ACCOUNT THE PHYSIOLOGY OF MUSCLE CONTRACTION AND THE ACTIVATION PATTERNS OF MUSCLE SYNERGIES. Gin McCollum and Lewis M. Nashner. Neurological Sciences Inst., Good Samaritan Hospital and Med. Ctr., 1120 N.W. 20th Avenue, Portland, OR 97209.

The actual mechanics of posture is determined by the physiological substrate. Many muscles are biarticular, so the effect of a muscle activation is the exertion of two torques on two joints. The interconnections of muscles with joints can be represented as a connection tensor, allowing the overall mechanics of one muscle or a set of muscles to be calculated in combination. Sets of muscles tend to act together in stereotyped spatial and temporal patterns. It is these patterns and not activations of single muscles which are simple in terms of CNS control of automatic postural corrections. Mechanics arising from physiology must relate the mechanics of single muscles to overall activation patterns and then physiologically observed activation patterns to motions of the body.

We are developing a physiological mechanics for application to stance on a moving platform. The task is to maintain an upright position under perturbations of the support surface.

The mechanics of the human leg in the forward plane, ankle, hip, and knee, can be calculated using three coordinates. The actual calculations performed by the CNS in walking and postural corrections can be duplicated by 1) computing in the same coordinates as are used physiologically, 2) making the same assumptions, for example about the effects of gravitation on the body, and 3) using the same organizational logic of computation. Coordinates are chosen from sensory and motor patterns found in subjects in a standing position in a gravitational field. Gravitation is represented as the drift from one stance position to another caused by the force of gravity; such a representation, rather than a more abstract physical one, might be expected to be found in CNS responses.

This biomechanical formulation has been used to analyze the postural corrections of both normal subjects and patients with movement disorders. Abnormal spatial and temporal activation patterns are found to match, mechanically, the functional disorders of patients.

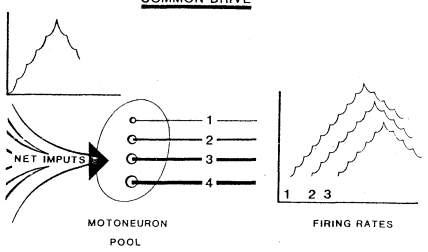
78.10 THE COMMON DRIVE OF MOTOR UNIT FIRINGS. C.J. De Luca and R.S. LeFever*. NeuroMuscular Research Lab., Dept. of Orthopaedic Surgery, Harvard Medical School, Children's Hosp. Med. Ctr., Boston, MA 02115.

Multiple channel myoelectric signals were detected from the First Dorsal Interosseous and Deltoid muscles of 13 normal subjects with varied physical skills during constant-force and force-varying isometric contractions performed up to 80% of maximal voluntary force. These were decomposed into their constituent motor unit action potentials using a computer-assisted procedure (LeFever and De Luca, IEEE Trans. Biomed. Engrg. 29, 149-157, 1982), allowing accurate calculation of the average firing rates of up to 8 concurrently active motor units.

All 232 motor units examined were found to have firing rates which fluctuated about an average value of approximately 1.5 Hz. Cross-correlations of the firing rates indicated remarkably high values (in some cases >0.9) with a virtual lack of time shift between these fluctuations. This communality of behavior was noted in both muscles, in all subjects, and in all contractions.

The persistent, apparently ever-present communality of behavior of the firing rates of concurrently active motor units during isometric contractions points to a common drive regulating the firing characteristics of motor units (see accompanying figure). A possible conceptualization is that the net excitatory and inhibitory input (supraspinal, segmental and infraspinal) to the motoneuron pool in the anterior horn has a simultaneous and common effect on all the motoneurons excited at any given time. The recruitment properties of a motoneuron are determined by the Size Principle, but once a motoneuron becomes active, its firing rate behavior is regulated by a common drive. Thus, when the level of excitatory inputs in the anterior horn increases, the firing rates of all active motoneurons increase in like fashion. Correspondingly parallel behavior is noted for increased inhibitory inputs (or decreased excitatory inputs). (Supported in part by Liberty Mutual Insurance Company).

COMMON DRIVE



79.1 FMRFamide MODULATION OF CONTRACTILE BEHAVIORS OF THE ISOLATED *APLYSIA* GILL. Sam Weiss*, Jeff Goldberg*, George I. Drummond* and Ken Lukowiak*. (Spon: Keith E. Cooper). University Biochemistry Group and Department of Medical Physiology, University of Calgary, Calgary, Alberta. T2N 1N4.

The neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH_2), discovered in the ganglia of the clam *Macrocallista nimbosa*, has diverse actions on various molluscan cardiac and non-cardiac muscles. Recent studies have demonstrated the occurence of FMRFamide in vertebrate and invertebrate nerve tissue and suggest a role in modulation of neuronal activity. The *Aplysia* gill, isolated from the central nervous system, contracts both spontaneously and reflexly in response to tactile stimulation. We have reported that the putative neurotransmitters serotonin and dopamine modulate *Aplysia* gill behavior and have provided evidence that cAMP may be involved in these processes. In the present study, our objective was to determine whether neuropeptides, such as FMRFamide, influence gill behavior and to examine possible molecular mechanisms. Isolated gills were perfused with artificial sea water (ASW) via cannulation of the afferent vein and contractions were measured with a force transducer; tactile stimuli were delivered with a mechanical tapper. All test substances were added at the indicated concentrations to ASW immediately prior to use. Perfusion of FMRFamide (1 nM-1 μM) resulted in a rapid onset of spontaneous contractions. This effect was dose-dependent. In addition,in most preparations FMRFamide potentiated gill reflex response to tactile stimulation. These effects were readily reversible. Other neuropeptides tested: methionine-enkephalin, arginine vasotocin and vasoactive intestinal polypeptide, at concentrations as high as 1 μM were without effect. Perfusion of the membrane-permeable cAMP analog p-chlorophenylthio cAMP (50-100 μM) resulted in spontaneous contractions and potentiated reflex responses of long duration. When examined in a particulate preparation of *Aplysia* gill, FMRFamide stimulated adenylate cyclase activity 2 to 3-fold over basal. Other neuropeptides tested, as well as the amino acids phenylalanine, methionine, and arginine were without effect with respect to enzyme activation. These findings demonstrate that FMRFamide may modulate contractile behavior of *Aplysia* gill and suggest that the contractile effects may be mediated by cAMP. These results and related studies currently underway with isolated heart and gut preparations suggest that FMRFamide may strongly influence a variety of physiological mechanisms in *Aplysia*.

(Supported by the Medical Research Council of Canada and Alberta Heritage Foundation for Medical Research Studentships to S.W. and J.G.)

79.2 IDENTIFICATION AND INTRACELLULAR RECORDING OF A BUCCAL GANGLION MOTORNEURON ACTIVATED BY APLYSIA EGG-LAYING HORMONE. Jeffrey L. Ram. Dept. of Physiology, Wayne State Univ., Detroit, MI 48201.

Aplysia egg-laying hormone, a 34 amino acid 4380 dalton peptide which has been purified and sequenced (Chiu et al., PNAS 76:6656), is synthesized and secreted by the bag cell neurons of the Aplysia abdominal ganglion.

Application of purified Aplysia egg-laying hormone (ELH) to isolated buccal ganglia activates a neuron having an axon in buccal nerve B4 (Stuart & Strumwasser, J. Neurophysiol. 43:499). The B4 axon activated by ELH sends excitatory activity to a radula closer muscle, I5 (Ram, Brain Res. 236:505).

Aside from its responsiveness to ELH, the ELH sensitive motorneuron can be identified by its location in the ganglion and the degree of facilitation of its input to I5. The neuron is located near several large neurons on the medial rostral surface of the buccal ganglia. Two large inputs to I5 turned on by stimulating nerve B4 exhibit different degrees of facilitation at moderate stimulation frequencies (1 - 2 Hz). In 12 out of 12 preparations, the unit showing less facilitation was the one turned on by application of bag cell extract or partially purified ELH. Cohen et al. (J. Neurophysiol. 41:157) previously identified the two major inputs to I5 as neurons B15 and B16. B16 has a medial rostral location adjacent to large cells B3, B4, and B5. The input from B16 to I5 facilitates less than that of B15. On the basis of these two criteria, I suggest that the ELH-sensitive motorneuron is probably the same as cell B16 of Cohen et al.

Intracellular recording from this neuron shows (a) that shortly after application of ELH-containing extracts, the cell begins to depolarize, (b) that action potentials activated by ELH-containing extracts rise off an apparently smooth pacemaker potential, (c) that no epsps driving these action potentials are revealed by hyperpolarizing the cell to block action potentials, (d) that the spiking frequency becomes temporarily higher following a hyperpolarizing pulse, and (e) that in three cells in which the membrane potential has been manually "clamped" to a fixed potential below threshold and conductance measurements have been made by passing hyperpolarizing current pulses, that input resistance increases in the presence of ELH-containing extracts. It seems likely that ELH is acting directly on this neuron, although these data do not rule out the possibility that slow or smoothly graded synaptic inputs are being turned on. The working hypothesis to explain depolarization accompanied by an increase in input resistance is that an ELH-sensitive K^+ channel is being closed.

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79.3 SUBSTANCE P MEDIATES CHANGES IN SENSITIVITY OF THE LATERAL EYE OF LIMULUS. Jorge R. Mancillas and Allen I. Selverston. Depts. of Neurosciences and Biology, Univ. of Calif. San Diego, La Jolla, California, 92093

The lateral eyes of Limulus contain a system of substance P immunoreactive fibers, which appear to modulate the photoreceptors' sensitivity to light. They were detected, and their distribution studied, using indirect immunocytochemical techniques and a monoclonal substance P antibody. These thin, efferent fibers travel up the optic nerve, through the lateral plexus and branch out profusely when reaching the ommatidia. Innervation seems to be extended to more than one component of the ommatidia, including the retinular and pigment cells. Immunoreactive staining could be abolished by absorbing the antiserum with as little as 1 μM synthetic substance P. No serotonin or enkephalin immunoreactivity was observed.

Radioimmunoassays with two different C-terminal antibodies confirmed the presence of substance P-like material in quantities of up to 18 ngs. per eye or 61.44 pgs. per μg. of protein. Gel filtration chromatography in a Bio-Rad P-4 column, followed by RIA, revealed that the immunoreactive material elutes in the same region than synthetic substance P.

It has been reported (Barlow,R.B. et al, Science 210:1037,1980) that the Limulus lateral eye undergoes regular circadian changes in sensitivity to light, displaying higher levels of sensitivity at night, and lower ones during the day. These changes are correlated with morphological changes of the photoreceptor complex and appear to be controlled by an efferent system of fibers, which resembles the substance P-containing one. Thus, we explored the possibility of substance P mediating those morphological and sensitivity changes by testing the effects of substance P application on 1) light-induced electroretinograms recorded from intact preparations and 2) the morphology of the cellular components of the ommatidia.

Substance P injections into the eye (10^{-9} to 10^{-7}m.) caused an increase in the receptors' response to light, which resembled that reported for efferent fiber stimulation. The latency, magnitude and duration of the increase was dose dependent. Control injections of saline or other neurotransmitters had no effect. Furthermore, incubation of excised eyes in synthetic substance P resulted in morphological changes that could underlie such increases in sensitivity. These changes included increased apperture and rhabdom diameter, contraction of the retinular cells and rhabdom and closer apposition of the lens and rhabdom, all of which would increase the angle of acceptance and the quantum catch.

We are now using intracellular recording and pharmacological techniques to elucidate the membrane and molecular mechanisms by which substance P induces the observed morphological and sensitivity changes.

79.4 ACTIONS OF THE SUBSTANCE P ANALOGS (D-PRO^2,D-PHE^7, D-TRP^9)-SUBSTANCE P AND (D-PRO^2,D-$TRP^{7,9}$)-SUBSTANCE P IN MYENTERIC GANGLIA OF GUINEA-PIG SMALL INTESTINE. P. R. Nemeth*, W. R. Ewart*, and J. D. Wood. Department of Physiology, University of Nevada School of Medicine, Reno, NV 89557.

The substance P analogs (D-Pro^2, D-Phe^7, D-Trp^9)-substance P and (D-Pro^2, D-$Trp^{7,9}$)-substance P are reported to be substance P antagonists in certain peripheral locations.[1,2] These substance P analogs were individually studied to determine their effects on the slow excitatory postsynaptic potentials (slow EPSPs) and the excitatory action of exogenously applied substance P on myenteric ganglion cells. Intracellular methods were used to record electrical activity of the ganglion cells in vitro. Slow EPSPs were evoked in neurons by electrical stimulation of presynaptic fibers that projected in interganglionic connectives. Substance P was applied to the neurons from fine pipettes by microejection with nitrogen pressure pulses of controlled amplitude and duration. The substance P analogs were applied in the superfusion solution. Electrical stimulation of the connections evoked slow EPSPs in AH/Type 2 myenteric neurons. Changes in neuronal excitability were evaluated by counting the numbers of action potentials evoked by intracellular injection of depolarizing electrical current pulses of constant amplitude and duration. Microejection of substance P onto the neurons mimicked the stimulus-evoked EPSPs. Neither of the substance P analogs (1-100 μM) in the superfusion solution suppressed the slow EPSP. Neither of the substance P analogs decreased the number of spikes evoked by the current pulses in the presence of substance P. Usually, the number of spikes evoked was increased. Occasionally, either of the substance P analogs increased the number of spikes evoked and mimicked the slow EPSP without concomitant microejection of substance P. We conclude that neither (D-Pro^2, D-Phe^7, D-Trp^9)-substance P nor (D-Pro^2, D-$Trp^{7,9}$)-substance P are antagonists of substance P at the myenteric ganglion cells. (Supported by NIH Grants NS17363 and AM26742.)

1. Holmdahl, G., Science, 214(4224):1029, 1981.
2. Leander, S., et al., Nature, 294(5840):467, 1981.

79.5 THROTROPIN RELEASING HORMONE MODULATION OF BARBITURATE ANESTHESIA, M. D. Hirsch* (Spon.: R. Esposito). Dept. of Psychology, Queens College of the City University of N. Y., Flushing, NY 11367.

Pharmacological and biochemical studies were performed in mice to characterize thyrotropin releasing hormone (TRH) antagonism (analepsis) of barbiturate anesthesia. Adult male CF-1_S mice received intracerebral ventricular administrations of 0.9% saline, or 10µg or 20µg TRH 1 min or 45 min before 50 mg/kg sodium pentobarbital intraperitoneally. Interinjection durations (IIDs) were manipulated within treatment subgroups in counterbalanced order across two repeated sessions. Half of each subgroup was tested either in morning or evening sessions. Pentobarbital anesthesia was measured as duration between loss-regain of righting reflex (3 times/60s); locomotor activity was measured as number of rectangles crossed (four 13.0 x 8.0 cm rectangles per cage) in 10 min. Results indicated that TRH is a potent dose-related analeptic at 1 min IID, although TRH's actions were markedly attenuated at 45 min IID. TRH did not stimulate locomotor activity of mice either before or after barbiturate. Neither diurnal nor sequential variations were observed in either paradigm. Spectrophotometric assays of regional brain pentobarbital indicate that TRH does not alter barbiturate disposition. Radioligand binding assays were performed by incubating synaptosomal membranes from regional mouse brain with 5nM of [^{3}H] TRH in the presence or absence of 50µM of either TRH or barbiturate analogues. Barbiturates effectively displaced TRH at highly dense and specific TRH binding sites of limbic forebrain and brain stem, and potentiated TRH binding at less specific cerebral cortex sites. The findings of this series of studies suggest: (a) TRH analepsis is dose-related via central infusions due to circumvention of problems of brain transport and enzyme catabolism; (b) temporal factors influence TRH analepsis; (c) TRH is not a central motor stimulant; (d) analepsis is probably not mediated by peptide alterations of brain barbiturate distribution; (e) TRH analepsis appears to be mediated at discrete TRH binding sites of mouse brain.

79.6 APPETITE REGULATING PEPTIDES IN CSF AND PLASMA OF ANOREXIA NERVOSA PATIENTS. D.L. Ross, R.F. Murphy*, R. Hitzemann, M. Berelowitz*, M. Maloney*, M. Farrell*. Departments of Neurology, Pediatrics, Surgery, Psychiatry and Medicine, University of Cincinnati, OH 45229.

Anorexia nervosa is an eating disorder of unknown etiology. The present study was done to determine whether an imbalance of appetite regulating peptides in CSF and plasma could account for the abnormal eating pattern. Beta endorphin (BE) stimulates feeding when infused intraventricularly. CSF insulin (IN) is a regulator of long term food intake and weight maintenance. Cholecystokinin 33 (CCK), pancreatic polypeptide (PP) and somatostatin (SS) are short term satiety factors which have actions both centrally and in the GI tract.

Simultaneous samples of lumbar CSF, alliquot 3-6 cc., and plasma were obtained under overnight fasting, recumbent conditions from anorectic, normal and obese female subjects characterized for nutritional status, anorectic behavior and subjective appetite. Specimens were saved at -70° until group assay by radioimmunoassay in triplicate. CSF data is presented in table.

	Subjects	BE pg/ml	CCK pg/ml	PP pg/ml	IN uU/ml	SS pg/ml
1	Anorexia	52	590	28	1.0	48
2	"	35	430	13	1.4	37
3	"	63	370	16	1.3	30
4	Anorexia-Bullemia	10	370	20	1.0	21
5	Anorexia	33	310	16	1.1	18
6	"	30	385	19	1.5	24
7	"	41	475	16	1.1	44
8	Anorexia-Bullemia	35	615	15	1.3	16
9	Anorexia	54	415	12	1.0	36
	Anorectics (mean)	39	440	17	1.2	30
10	Normal	29	295	20	1.2	30
11	Obese	159	520	13	1.6	31
12	Prader-Willi	380	285	24	1.0	12

Plasma peptide levels showed no systematic correlation with CSF values. Plasma insulin showed the expected correlation with body weight. The anorexia-bullemia and the Prader-Willi subjects showed high plasma CCK values (2000 pg/ml).

No significant differences between CSF values of anorexia and control subjects was noted on BE, PP, IN and CCK assays. The anorexia-bullemia subjects had relatively lower SS as did the Prader-Willi subject, suggesting that the anormally increased eating might be related to a deficiency of that hypothalamic satiety factor.

79.7 EFFECT OF INTRACEREBROVENTRICULAR INSULIN ON PLASMA CATECHOLAMINES OF RATS. E. C. Lotter* and R. G. Campbell* (SPON: R. C. Emerson). Dept. of Medicine, Univ. of Rochester, Rochester, New York 14642.

Insulin infused into the lateral cerebral ventricle of baboons decreases food intake and body weight with no reliable effect on basal plasma immunoreactive insulin (IRI). Moreover, injections of norepinephrine (NE) into the hypothalamus elicit an immediate rise in plasma IRI. The NE-stimulated insulin response is similar to that reported when rats eat a carbohydrate meal. A first phase, independent of absorbed nutrients (early insulin response or EIR) and a second more prolonged insulin response dependent on an increase of blood nutrient levels. The present study investigates the relationship between the peptide hormone insulin and its effect on catecholamines.

Rat insulin was infused into the lateral cerebral ventricle of free-moving unanesthetized Wistar rats for 1 h/day. CSF infusions (15 λ) contained either artificial rat CSF or CSF plus rat insulin (100 mU). Infusion of CSF alone for two weeks had no reliable effect on either epinephrine, norepinephrine or glucose levels. Addition to the infusate of the insulin for the same period of time resulted in a significant decrease of plasma epinephrine and norepinephrine ($p < 0.05$). No change in plasma glucose levels was observed. These data are the first report of intracerebroventricular infusions of insulin altering plasma catecholamines without any changes in glucose levels and may account for the observed decrease of food intake and body weight associated with insulin infusions.

79.8 THERMOREGULATORY EFFECTS OF CENTRALLY ADMINISTERED HIBERNATION "TRIGGER" AND BOMBESIN IN A HIBERNATOR. M. Steiner and E. B. Pivorun*. Department of Zoology, Clemson Univ., Clemson, SC 29631 and P. R. Oeltgen*. V.A. Hospital, Lexington, KY 40507.

A number of peptides or proteins have recently been isolated from animals while in a state of depressed metabolism (i.e., hibernation). Antabolone, a partially purified polypeptide extracted from the brains of hibernating ground squirrels has been shown to cause a marked decrease in oxygen consumption and body temperature (T_b) following intravenous (i.v.) administration in rats and mice. Hibernation "trigger" (HIT), a blood-borne substance found in hibernating rodents has been shown to induce hibernation out of season in ground squirrels following i.v. administration, while in the monkey intraventricular administration resulted in hypothermia and hypophagia. The following experiment was performed to assess the thermoregulatory effects of centrally injected (HIT) and bombesin (B) in the thirteen-lined ground squirrel, a hibernator.

Indwelling bilateral 23-ga cannula guide tubes and a thermocouple entry tube were stereotaxically placed just above the pre-optic region of the hypothalamus. Injection cannulae (28-ga) were lowered to a depth of 0.5 mm below the tips of the guide tubes. Temperatures were recorded continously for one hour prior to remote bilateral injection of 0.5 ul of peptide solutions , and for two hours thereafter. Each group of animals received only one treatment, which consisted of either CSF (control), 10-100 ug (HIT), or 1 ug (B). The experiments were conducted at ambient temperatures of 5°C and 20°C. Animals were sacrificed and cannula placement was verified by histological inspection.

Centrally injected CSF imparted little change in body temperature, although a slow tendency toward hyperthermia was evident. The effect of 10-100 ug of centrally administered (HIT) did not appear to be significantly different from the CSF control group. In comparison, 1 ug of (B) elicited a maximum fall in T_b of 1.16±.32 °C at 20°C and 2.73±.43 °C at 5°C. The (B)-induced hypothermia reached a maximum at 30-45 minutes post-injection and persisted for up to two hours.

The results lend support to the proposed role of (B), in temperature regulation. Other workers suggest that (B) acts within the hypothalamus to disrupt temperature regulation. In experiments reported here, it would seem that (HIT) does not act within the hypothalamus to regulate temperature. The absence of this effect does not preclude action(s) elsewhere within the CNS. The hippocampus has been implicated in thermoregulation as well as in the neural control of hibernation.

79.9 INHIBITION OF GASTRIC ACID SECRETION IN RATS BY CORTICOTROPIN RELEASING FACTOR (CRF). Y. Taché and J. Rivier*. Pediatric Research Center, Ste-Justine Hospital, University of Montreal, Montreal, Canada, H3T 1C5 and Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Our previous studies suggested that some endogenous neuropeptides may participate in the brain control of gastric secretion based on their ability to markedly influence gastric secretion when injected into the brain (Taché et al., Peptide 2, suppl. 2: 51, 1981). The recently characterized and chemically synthetized CRF, which has been shown to elicits endocrine and autonomic changes characteristic of stress response (Vale et al., Science 213: 1394, 1981; Brown et al., Proc. Soc. Natl. Acad. Sci., in press) lead us to evaluate its influence on gastric acid secretion. Twenty-four hour-fasted male rats under light ether anesthesia were injected intracisternally either with saline or various doses of CRF, and the pylorus was ligated. Conscious animals were decapitated 2 hours post injection. CRF (10 µg) inhibited by 80% gastric acid output by reducing gastric secretory volume and gastric acid concentration, and increased plasma gastrin levels. Lower dose (1 µg) significantly reduced gastric acid concentration and acid output by 44% but did not modify gastric volume. Acute adrenalectomy completely prevented the effects of CRF (10 µg) on gastric secretory volume, acid concentration and output. Chemical sympathectomy produced by administration of guanethidine (40 mg/kg, i.p. once a day for 5 weeks), hypophysectomy (-5 days) or naloxone (5 mg/kg, s.c.) did not modify CRF (10 µg) induced inhibition of gastric acid output. These results demonstrated that intracisternal injection of CRF inhibits gastric acid secretion in rats. Gastric response to CRF is unrelated to associated changes in pituitary hormones (opioid peptides, ACTH) or gastrin secretion but is dependent upon adrenal secretion probably through activation of adrenomedullary component of the sympathetic nervous system.

79.10 STRIATAL SOMATOSTATIN: IMMUNOHISTOCHEMISTRY AND EFFECTS ON DOPAMINERGIC TRANSMISSION. M.F.Chesselet*, T.D.Reisine, J.Glowinski* and A.M.Graybiel. (SPON: F.O.Schmitt). Groupe NB, College de France, Paris 75231, France and Dept. of Psychology, Mass. Inst. of Technology, Cambridge, MA 02139

In immunohistochemical studies, Sternberger's PAP method was applied in 3 cats to analyze the distribution and morphology of striatal somatostatin-immunoreactive (SOM) neurons and in 3 cats, FITC immunofluorescence was combined with Kuypers fast blue (FB) retrograde tracing to determine whether these SOM neurons projected to the substantia nigra (SN). In both PAP and FITC preparations, SOM neurons in the caudate nucleus (CN) tended to occur in groups of ca.3-20 cells with few or no SOM cells in between. Within these groups, SOM cells were not evenly dispersed. Of 311 SOM cells measured in PAP preparations, 90% had soma diameters of 8.5-20± 2µm, 10% a major diameter greater than 22µm(8cells>30µm). At least half the 971 SOM cells studied in detail were typified by having a stocky dendrite which tended to bifurcate ("snail cells"). Most well impregnated cells had varicose dendrites. In the 3 cats with FB injections centered in the SN, dense FB cell-labeling appeared in sections of the CN processed both for FB and FITC(SOM). Though SOM (FITC) and FB-positive cells often lay next to one another, of 2064 SOM neurons identified by FITC, 2048 did not contain FB; only 16 appeared double labeled and in these, superposition of singly labeled cells could not be definitely excluded. We conclude that SOM cells constitute a considerable population of medium sized caudate neurons and that unless a major axon collateralization is present in these neurons, at most only a few, if any, project to the SN.

As a step toward defining the function of somatostatin in the striatum, the effect of this peptide on the release of tritiated dopamine (^{3}H-DA) was investigated both in vitro in rat striatal slices and in vivo by means of push-pull cannulae implanted in both CN and both SN of halothane anaesthetized cats. In both cases the tissue was continuously superfused with a medium containing ^{3}H-tyrosine and the newly formed ^{3}H-DA was estimated in serial fractions after biochemical separation. In vitro, somatostatin ($3x10^{-10}$ to $3x10^{-7}$M) increased the spontaneous calcium-dependent ^{3}H-DA release and this effect was prevented by $5x10^{-7}$M tetrodotoxin. In vivo, somatostatin (10^{-7}M) applied for 30 min in one CN not only locally increased ^{3}H-DA release but also induced a prolonged decrease of the ^{3}H-amine outflow in the contralateral CN. No changes of DA release were observed in the two SN. These results suggest that striatal somatostatin may intervene in the intrastriatal regulation of DA release and, through an action on striatal efferents, influence the activity of the contralateral nigrostriatal neurons. Supp. by NSF Grant BNS 81-12125, NASA Grant NA62-124, INSERM(ATP 587890), DRET(78004),Rhone-Poulenc S.A.

79.11 MODULATION BY VASOACTIVE INTESTINAL PEPTIDE(VIP) OF SEROTONIN$_1$ RECEPTORS IN THE MALE RAT BRAIN. W.H. Rostene (Rotsztejn), C.T. Fischette and B.S. McEwen. The Rockefeller University, New York, NY 10021.

The vasoactive intestinal peptide (VIP) has been located in various structures of the rat brain, but few actions of the peptide have been reported as yet. Since VIP might interact with classical neurotransmitters in the CNS as it does in the periphery, we investigated whether VIP can modulate serotonin$_1$ ($5HT_1$) receptors in brain areas which contain various amounts of VIP and 5HT, i.e., the hippocampus (dorsal and ventral); hypothalamus (anterior, mediobasal and posterior), parietal cortex and midbrain (raphe area). Binding assays were performed on crude membrane preparations as previously described (Biegon and McEwen, J. Neurosci. 2:199, 1982). Tissue was preincubated for 45 min at 25°C in the presence or absence of VIP and/or bacitracin in 50mM Tris buffer, pH 7.6, and then incubated with various concentrations (0.6-4.4nM) of tritiated 5HT (26.4 Ci/mmol) in Tris-Pargyline-ascorbate buffer, with or without 5µM cold 5HT for estimating nonspecific binding. Total and non-specific binding were measured by filtration through Whatman GF/B filters using a cell harvester. The presence of bacitracin alone (20µM), an inhibitor of peptidase activity, significantly increases the K_d for $5HT_1$ receptors in almost all the structures tested. Scatchard analysis indicates that VIP (0.2µM) significantly ($p < 0.01$) increases both K_d (3.8±0.3 bacitracin vs 6.0± 0.4 bacitracin + VIP) and number of binding sites for $5HT_1$ (462±22 vs 747±10) in the dorsal hippocampus and not in the ventral hippocampus. Other areas were not affected. This effect of VIP is not observed when bacitracin is omitted; moreover, the presence of calcium (4mM) which dramatically increases the number of binding sites for $5HT_1$ does not alter the efficacy of VIP in the dorsal hippocampus. The present data suggest a possible interaction between VIP and $5HT_1$ receptors in the dorsal hippocampus, a structure important for its behavioral and neuroendocrine role related to glucocorticoid action.

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79.12 CNS VASOPRESSIN MODULATION BY ETHANOL: POSSIBLE IMPLICATIONS FOR MEMORY FUNCTION. R.E. Brinton, P.P. Deshmukh, S. Hsiao, H.I. Yamamura. Dept. of Pharmacology, Arizona Health Sciences Center & Dept. of Psychology, University of Arizona, Tucson, AZ 85724.

Alcohol has been shown to reduce plasma levels of vasopressin (AVP), a hypothalamic neural peptide (Rubinin et al., 1955). In addition, alcohol intoxication is associated with mild to severe memory deficits in human subjects (Goodwin et al., 1969) and in experimental animals (Jacobsen, 1975). In the past decade, it has been shown that vasopressin, in addition to its endocrine function, is involved in memory processes (DeWied, 1971; Koob et al., 1981). Because of the effect of alcohol on plasma vasopressin levels and memory, and the relationship between vasopressin and memory processes, we investigated the effect of alcohol on CNS levels of this neural peptide.

Male Wistar rats were treated with an intraperitoneal injection of ethanol (1.6 g/kg, 20% w/v). Control animals received an equal volume of 0.9% NaCl. Animals were sacrified 15 min post-injection and the brain immediately removed. Vasopressin content of tissue extracts was measured by a nonequilibrium radioimmunoassay according to the technique described by Brinton et al. (1982).

Acute administration of ethanol resulted in greater concentrations of vasopressin in the paraventricular, supraoptic, and suprachiasmatic nuclei of the hypothalamus and in the pituitary. Differences were not found in the septum, corpus striatum, thalamus, hippocampus, amygdala or substantia nigra.

These data are consistent with a dual role of vasopressin. The dehydrative effect of ethanol may have resulted in an increased production of vasopressin. The newly synthesized AVP was subsequently transported to the neurohypophysis for release into the periphery.

It is of interest that a change in AVP concentration was not observed in brain regions receiving vasopressin innervation outside the neurohypophseal system. Deficits in memory function produced by ethanol may be mediated through inhibition of vasopressin release in the CNS. The data reported above, showing no difference in AVP levels at the terminals, are consistent with such a possibility. Release studies to investigate this hypothesis are currently in progress.

79.13 PEPTIDERGIC TRANSMISSION AT A SKELETAL NEUROMUSCULAR JUNCTION. M.E. Adams and M. O'Shea. Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

Neuropeptide proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) is present in an identified leg motoneuron in Periplaneta americana, the slow coxal depressor (Ds) neuron, and proctolin-immunoreactive innervation is found on some coxal depressor muscles (O'Shea & Bishop, J. Neurosci., 1982, in press). This suggests that proctolin is a neuromuscular transmitter of Ds. To test this idea we have attempted: 1) to show specific association between proctolin innervation and the Ds neuron; 2) to measure the release of proctolin during stimulation of Ds; 3) to determine mechanical and electrophysiological responses of the slow depressor muscles to both proctolin and Ds stimulation; and 4) to investigate the proctolin-sensitivity of skeletal muscles which do not receive innervation by proctolin-containing neurons.

Proctolin-immunoreactive terminals are found on the slow coxal depressor muscles (muscles 177d and 177d') which are innervated by the Ds neuron. Fast coxal depressor muscles that are not innervated by Ds do not have proctolin-immunoreactive junctions. Evidence for proctolin-releasing terminals on muscle 177d is provided by a demonstration of release of proctolin-like bioactivity during nerve stimulation (50 Hz, 10 mins) in an isolated nerve-muscle preparation.

Muscle 177d is highly sensitive to proctolin. At 10^{-9} M, proctolin causes the slow development of a sustained tension (30 secs to peak). The response outlasts the presence of proctolin, often for minutes. The sustained tension increases with concentrations up to about 3g at 10^{-7} M. At this concentration, twitch contractions are also produced. No mechanical response to proctolin has been obtained from fast coxal depressor muscles that do not receive innervation from Ds and that do not receive proctolin-immunoreactive terminals. The mechanical effects of applied proctolin are similar to the effects of Ds stimulation. A slow, sustained tension, which is proportional to stimulation frequency (5-50 Hz), is produced, and the neurally-evoked tension outlasts motoneuron activity, often for minutes. The electrophysiological response to proctolin at 10^{-7} M in muscle 177d is a slow, reversible increase in input resistance (from about 1 MΩ to 2 MΩ). This is often accompanied by a 3-5 mV membrane depolarization and a reduced threshold for active responses to injected current. These effects are correlated with tension development. We are comparing these electrophysiological effects to those produced by the proctolin-containing Ds motoneuron.

Our evidence suggests a transmitter role for a peptide at a skeletal neuromuscular junction.

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80.1 CONTRIBUTION OF HEAD MOVEMENT TO THE PROJECTIVE AIMING ACCURACY IN ADULTS. C. Bard, J. Paillard and M. Fleury. Lab. of Physical Activity Sciences, Laval Univ., Québec, G1K 7P4, Canada.

Accuracy of aiming at target has been shown to be significantly improved when the head was allowed to move as compared with aiming in fixed head condition, specially while aiming at eccentric targets (Roll, R., Bard, C., Paillard, J., 1981). Factors contributing to this improvement are still debated. Combined triggering of eye and head movements appeared to be the rule in animals and children. However, in human adults different strategies may be observed, leading to a stable typology. Some consistantly move their head in manual aiming, even for small target eccentricity (head movers or cephalodynamic subjects = C.D.), while others hold their head stable during acquisition of the target at rather large eccentricity (eye movers or cephalostatic subjects = C.S.). It is therefore logical to predict that C.D. will be more disturbed in fixed head aiming than in free head condition, whereas C.S. will not be strongly affected. The aim of the research was to analyze the accuracy of directional aiming, according to the head conditions (fixed, free of lined up), subject's characteristic (C.S. or C.D.), and target eccentricity. Twelve subjects (males and females) were selected according to their dominant strategy while aiming at a visual target; six were identified at C.S., and six as C.D. Subject, holding a joy stick, performed a direct aiming without visual feedback on a vertical target. Targets were localized at 0, 10, 20, 30 and 45° in the right and left visual fields (a total of 9 targets). For each trial, RT and MT of the arm and angular errors in aiming were collected. Results showed that, C.D. were significantly less accurate in the fixed head condition, whereas C.S. were not affected. In terms of constant errors however, both groups improved significantly on free head condition compared to fixed head condidion. With head lined up, errors for both groups tended to shift in the direction of the head movement, even more so for the C.S. group. The contribution of head movement to the accuracy of aiming was therefore clearly established: it appeared to correspond to the more primitive form of eye-head coordination. C.S. have learnt to calibrate their aiming program without the instrumentality of the head, but were nevertheless able to use it accurately when instructed to do so. Conversely, C.D. did not learn to coordinate their program without head movement and were severely disturbed when deprived of this assistance.

80.2 EYE-HEAD COORDINATION IN MAN TO VISUAL TARGETS WITHIN AND BEYOND THE OCULOMOTOR RANGE. M. Volle*, D. Guitton, F.Jean*, and C.H. Nadeau* (SPON: R.Chase). Montreal Neurological Institute, McGill University, Montréal, Canada H3A 2B4

Two strategies may be used to bring a suddenly appearing visual target onto the fovea. In the saccade attenuation (SA) strategy a saccadic eye movement is programmed to acquire the target without the aid of a head movement. Any head movement occurring during the saccade attenuates, using the vestibulo-ocular reflex (VOR), the saccade amplitude by an amount equal to the head displacement. In the vector addition (VA) strategy the saccade and the head movement are separately preprogrammed such that the vector addition of both movements (i.e. the gaze) brings the eyes onto the target. The VOR must be switched off in this condition. Monkeys have been shown to use the SA strategy but whether humans use it too has not been conclusively shown. The object of the experiments was to determine which strategy is used by humans when they orient to targets within and particularly beyond their oculomotor range (OMR). In this latter case, if the SA strategy were used a saccade larger than one that can be physically made would have to be programmed by the nervous system. Methods: Five human subjects in a dim environment were required to fixate a small central LED and to orient, when this light came off, to a small visual target that suddenly and randomly appeared in their peripheral visual field. Targets were distributed in the horizontal plane in 10° intervals beginning at 20° to either side of center and ending at 70°. Target presentation lasted 3 sec. Eye movements were measured using EOG. Heads were fixed securely to a helmet which was attached via a universal joint to a vertical shaft equipped with a brake. Saccade characteristics were studied in three experimental conditions: 1. When the head was mechanically restrained; 2. When the head was free to move; and 3. When the head was unexpectedly braked a few milliseconds before the intended head movement. In all three conditions the only instruction given to the subject was "look as fast as possible at the suddenly appearing light". Results: Gaze shifts within the OMR (<55°). In this condition the amplitude of saccades accompanying the braked head movements were about equal to target eccentricity and were larger than those accompanying a head free movement to the same target. Thus the SA strategy holds. Gaze shifts beyond the OMR (>55°). Many saccades accompanying braked movements were larger than those in the head free condition and brought the eye to its mechanical limit which was often beyond the limit of voluntary movements. The SA strategy applied in these cases. However, in a number of trials in the same subjects saccades associated with braked movements were well within the OMR (30-35°) and were not driven to saturation. Furthermore in the head free case these small amplitude saccades did not bring the gaze onto the target and the VOR was switched off until the gaze reached the target. In these cases the VA strategy applied and the "switch" controlling VOR intervention appeared to be controlled by the required gaze position.

80.3 EYE-HEAD COORDINATION IN THE CAT. D.Guitton, R.M.Douglas* and M.Volle*. Montreal Neurological Institute and Aviation Medical Research Unit, McGill University, Montreal, Canada H3A 2B4.

In monkey a saccade is programmed to take the eye to a target irrespective of whether the head moves or not. If the head turns simultaneously, the saccade is correctly reduced in size (to prevent gaze overshoot) by the vestibulo-ocular reflex (VOR). This will be called the saccade attenuation (SA) strategy. Cats have an oculomotor range (OMR) limited to about +25° , but their field of view extends to about +70°. If they use the monkey strategy to acquire targets lying beyond +25° their brain must program saccades that cannot be physically made. We have studied horizontal gaze shifts within and beyond the OMR made spontaneously by 5 alert cats. Heads were totally unrestrained and both eye and head movements were measured by the magnetic search coil technique. Results: (1) General. Qualitatively similar gaze shifts of all sizes up to at least 50° were accomplished with a single saccade and a saccade-like head movement. Saccades without a head movement were very rare. Almost all saccades began after (mean latency 30 msec) the head started. This differs from Blakemore and Donaghy (J. Physiol. 300: 317-335, 1980). Most saccades started within 2° of center and the slow phase compensatory movement (VOR gain = 1) returned the eye there during the latter part of the head movement. (2) Head movements. Head amplitude equalled gaze amplitude. Head movement trajectories were stereotyped with peak velocities being highly correlated with amplitude. Large peak velocities (500deg/sec) were reached. (3) Eye-re-head, and gaze movements. For a given amplitude the peak velocity of the saccade was faster when the head was allowed to move than when it was fixed. However, despite scatter in the plots, the peak velocity of a gaze movement of a given amplitude within the OMR tended to be the same whether the head was fixed or free. This supports, but does not prove, that the SA strategy is used for gaze displacements within +25°. Beyond +25° gaze velocity saturated but since this is outside the OMR, no comparison with head fixed saccades could be made. Saccade amplitude was linearly related (slope 30%) to head amplitude for head amplitudes less than 25° to 30°. The saccade amplitude was constant (10-12°) for larger head movements. This suggests a different strategy is used for gaze shifts beyond the OMR. One possibility would be to preprogram separate eye and head movements. This "vector-addition" (VA) strategy requires (a) a pause in the VOR signal during saccades to allow the head movement to influence the gaze shift and (b) the stereotyped head trajectory which we have observed. To truly distinguish between the SA and VA strategies it is necessary to perturb the head movement trajectory during the accompanying saccade. We are doing this in trained cats. Preliminary results suggest that when the head is unexpectedly braked before small intended gaze shifts the saccade attains the target. For large gaze shifts, beyond the OMR, the saccade amplitude in the braked movement remains at 10°-12°. The results suggest the cat uses the SA and VA strategies for targets within and beyond the OMR respectively.

80.4 ARE RETICULAR NEURONS IN THE PERIABDUCENS AREA MEDIATING HORIZONTAL EYE POSITION SIGNALS TO DORSAL NECK MUSCLES OF THE CAT ? A. Berthoz*, P.P. Vidal*, J. Corvisier*. Lab. Physiol. Neurosensor. CNRS. Paris (France).

Previous results (Vidal P.P. et al, Exp. Brain Res., 46 3:448-453,1982) have demonstrated that dorsal neck muscle activity in the alert head fixed cat is related to the horizontal component of eye position towards the ipsilateral side with a threshold corresponding roughly to the mid-position of the eye in the orbit. Vestibulo-spinal neurons which carry both vestibular and eye movement signals and terminate both in the abducens nucleus and spinal cord (Yoshida K. et al, In Progress in Oculom. Res. Fuchs A, Becker W, 371-378, 1981) and reticulo-spinal neurons in the periabducens area which have been shown to project to cervical segments could mediate these effects. To test this latter hypothesis, experiments were performed in alert head fixed cats. Eye movements were measured by the search coil technique. Neuronal activity was recorded extracellularly with glass microelectrodes. Neurons were localized by stereotaxic coordinates and their position with respect to the antidromic field potential profile of the abducens nucleus. EMG of longuissimus capitis, obliquus capitis and splenius muscles was recorded. Vestibular stimulation was produced by a turn table. 12 reticular cells located below the abducens nucleus (1 to 3.5mm below the center of the nucleus. L : 1 to 1.5mm - A.P: 5.3 to 7.2mm in stereotaxic coordinates) were found to have a firing rate closely related to both neck EMG and horizontal component of eye movement during spontaneous saccades. Tonic (5) as well as burst tonic (5) and phasic (2) cells were encountered. They all fired only during eye movement towards the ipsilateral side. Their threshold was measured to be around the mid-position of the eye in the orbit. During vestibular stimulation the firing rate was also related mainly with eye movement and neck EMG. However, in some cells a head velocity signal was clearly added to the eye movement signal.We conclude from these preliminary results that an horizontal gaze generator (possibly the prepositus nucleus?) produces a common orienting signal for the head and eye during both saccades and nystagmus. The reticular neurons studied here could mediate the transmission of this signal to neck motoneurons.

80.5 NECK MUSCLE ACTIVITY EVOKED BY SUPERIOR COLLICULUS STIMULATION IN THE ALERT CAT. A. Roucoux*, M. Crommelinck*, C. Veraart*** and A. Al-Ansari* (SPON: M. David). Lab. of Neurophysiology, University of Louvain, 1200 Brussels, Belgium.

It has been shown (Guitton et al., Exptl Brain Res., 39: 63-73, 1980), in the head fixed alert cat, that Superior Colliculus (S.C.) electrical stimulation evokes two patterns of activity in the contralateral Biventer Cervicis muscle. In the rostral part of this structure, evoked contraversive retinotopic saccades are accompanied by a progressive increase of the activity in the contralateral Biventer, proportionally to eye displacement beyond a well-defined threshold. This relation of Biventer activity with eye position also exists during spontaneous exploratory or vestibular eye movements (Roucoux et al., in Progress in Oculomotor Research, Fuchs and Becker eds, pp. 129-135, Elsevier, 1981). More recently, Berthoz et al. (Exptl Brain Res., in press) have shown that this relation is particularly strong in the small neck muscles. The goal of the present study was to investigate the effect of S.C. stimulation on various neck muscles, as a part of a series of experiments aiming at clarifying their role in gaze control.

Activity of 12 neck muscles, on each side, evoked by S.C. microstimulation, was recorded by chronically implanted bipolar electrodes together with eye movements (coil technique) in alert head fixed cats. Our results showed that, in addition to the tonic discharges already described, S.C. stimulation yielded, in many contralateral muscles, a phasic discharge with a latency of 20 to 40 ms, slightly preceding or accompanying the evoked saccade. The intensity of these phasic discharges was related to eye deviation: for two similar amplitude saccades, the more eccentric one was accompanied by a larger burst. In the homologous muscle on the other side, this pattern of discharge was reversed. For a given muscle, there was a difference between the eye position thresholds for tonic and phasic discharges: the phasic component appeared earlier in a sequence of successive evoked saccades. Moreover, the thresholds differed from one muscle to the other.

These observations suggest that, in a way similar to the caudal part of cat's S.C., the rostral part is directly involved in head movement command. This command is modulated by eye position in the orbit.

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80.6 HEAD AND EYE CONTRIBUTIONS TO GAZE DRIFT OF MONKEY IN DARKNESS. H. Maldonado and M. Schlag-Rey. Dept. of Anatomy and B.R.I., U.C.L.A., Los Angeles, CA 90024.

In complete darkness, with the head restrained, a monkey often develops a slow ocular drift. Such drift is mostly unidirectional, independent of the position of the eyes in orbit, and remains so over months. When all head restraint is removed, a comparable gaze drift may occur. However, this drift is not exclusively produced by slow eye drift in a still head: at times, it also results from slow head drift, whether or not the eyes move. This observation prompted us to compare gaze drift in (1) the head-fixed and (2) the head-free condition, and in the latter, to assess the respective contributions of head and eye movements.

Gaze and head position were recorded in macaca nemestrina, with two search coils, one implanted around the eye, the other permanently mounted above the orbit. Eye position was obtained by electronically subtracting the head from the gaze signal. Additional data on eye drift alone were obtained from monkeys implanted with EOG electrodes. Gaze, head and eye signals were displayed on a CRO and a polygraph record and were stored on magnetic tape for subsequent computer analysis. The animals were observed through an infra-red sensitive, close-circuit video-system.

(1) In the head-fixed condition, dark-evoked gaze drifts (=eye drifts) occurred with variable latencies (1-50 s), durations (up to 15 s between saccades) and velocities (0.5^{o}/s - 3^{o}/s). (2) In the head-free condition, head movements perfectly compensated by eye movements often delayed or possibly masked a gaze drift which was seen only during periods of relative quiescence. When it occurred, its characteristics (direction, velocity and duration) were similar to those of the gaze drift observed with head-fixed. Gaze drift with head-free was caused interchangeably by eye drift alone, head drift alone or both. There were instances of perfectly linear gaze drifts consisting of an initial head drift instantaneously replaced by eye drift.

Since gaze drift had the same characteristics in both head-fixed and head-free animals and furthermore, since head and eye drifts in the same direction were never seen to increase the velocity of gaze drift, the signal responsible for the drift may be a gaze signal that can drive alternately either head or eyes. (Supported by USPHS Grant EY 02305)

80.PO CONVERGENCE INITIATED SACCADIC FLUTTER: A NORMAL INTRINSIC CAPABILITY, J. R. Hotson. Depart. of Neurol. Stanford Univ. Sch. Med., Stanford and Santa Clara Valley Med. Ctr., San Jose, CA.

Ocular flutter, as defined by eye movement recordings, consists of recurrent saccades oscillating in opposite directions with no pause between the end of one saccade and the beginning of the next. In humans spontaneous, large amplitude, flutter is considered abnormal though it can occur normally on a minature scale during visual fixation. Occasional individuals, however, can voluntarily produce bursts of fluttering saccades, traditionally called voluntary nystagmus. This observation has been considered an oddity of little significance and may be a trait that is dominantly inherited with variable penetrance.

Alternatively one model of saccade generation proposes that an intrinsic capability to generate flutter exists in both monkey and man. The alternating saccades that compose flutter may be produced by a continuous push-pulling between opposing, high-gain, pontine burst neurons. For these reasons it seemed worthwhile to determine whether voluntary flutter was an inherited peculiarity or an intrinsic, usually underdeveloped, ability of the human saccade system.

Five subjects with known voluntary nystagmus were examined with a purkinje image eyetracker to see if there was a genetic marker in the structure of visually-guided or flutter saccades, or in the configuration of microsaccades. Visually-guided saccades had normal peak velocity-amplitude curves. Flutter saccades plotted along the same curve as visually-guided saccades for each individual. The mean amplitude of microsaccades during visual fixation was 10.1 min arc, S.D. 2.8 for subjects with voluntary nystagmus compared to 9.5 min arc. S.D. 3.1 for 25 control subjects. The mean frequency of microflutter during fixation was 4.7/min; S.D. 6.7, (range 0-15/min) for subjects with voluntary nystagmus and 2.1/min; S.D. 3.2 (range 0-9/min) for control subjects. There was no significant difference between these values.

Next the saccade system of five normal control subjects was challenged to produce voluntary flutter. Four of five control subjects learned to produce bursts of fluttering saccades. The frequency, amplitudes and durations of flutter as well as the peak velocity-amplitude plots of flutter saccades were similar to subjects with voluntary nystagmus and indicated that flutter consists of recurrent complete saccades rather than saccades interrupted in midflight. All but one subject initiated flutter with a convergence movement suggesting that disconnecting horizontal versional eye movements increases an inherent instability in the saccade generating systems. (Supported by NEI grant #EY03387 and the Institute for Medical Research, San Jose, CA).

80.PO DO SACCADIC AND VESTIBULAR SIGNALS SUM? James H. Fuller Dept. of Oral Anatomy, Univ. of Ill. Med. Ctr. Chicago IL 60612

When mammals orient to a peripheral visual stimulus, the patterns of eye movement (**EM**) and head movement (**HM**) are different: the head moves only in the direction of the stimulus, whereas the first EM is towards the stimulus (a forward saccade, or **FEM**) and is followed by a counterrotary EM (**CEM**) which is usually equal and opposite the HM. In higher primates (monkey and man), eye-head coordinated movements toward targets located <40° in the periphery consist of a single FEM which sums with the HM (due to vestibular afferent stimulation) followed by a vestibularly driven CEM; the gaze is a single angular step.

In lower mammals, such as cat, FEM's and CEM's alternate several times if the target is eccentric by >20°; the gaze is a series of steps or a staircase. Staircase gaze shifts appear in primates if the target is >40°; in both species the eccentricity at which the step-staircase transition occurs is roughly the same as the oculomotor range (maximum excursion of the eyes in the orbit, or **OMR**).

In cats, it has been proposed that gaze shifts within the OMR are accompanied by summation. Cats moving their heads between targets were interrupted by an electronic brake attatched to their head holder; there was little evidence of summation, and if present, was seen only during the first FEM. Comparable data has been obtained in the rabbit. Employing the same test in the bush baby (a primitive primate) revealed summation even with gaze shifts of >80°; the OMR of this species is 25°.

This and other data suggest that the OMR is not related to the extent of summation; extensive analysis of the cat suggests an internal OMR, which is varied dependent on the task and strategy of movement. Whether the first FEM is summed with HM apparently reflects the range of the internal OMR at any given moment and does not necessarily reflect the physical OMR of the species (Roucoux, et. al., Exp. Brain Res. 39: 75-85, 1980); rather, it reflects whether a gaze shift will be in eye (summation) or head (no summation) coordinates.

81.1 INITIATION OF SMOOTH PURSUIT TO MOVING STIMULI AT DIFFERENT RETINAL ECCENTRICITIES IN THE MONKEY. S.G. Lisberger, W.T. Newsome, L. E. Westbrook*, and R.H. Wurtz. Dept. Physiol. and Div. Neurobiol. UCSF School of Medicine, San Francisco, CA 94143, and Lab Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20205

Smooth pursuit allows primates to rotate their eyes smoothly so that the fovea remains pointed at a small, slowly moving object. Although it is known that image movement is important for pursuit, little is known about the visual properties of an image that make it effective. We have begun to address this issue, studying first the properties of stimuli that can initiate pursuit; here, we report the effect of stimulus eccentricity.

Eye movements were recorded with the magnetic search coil in three monkeys. A bright, circular target was presented in discreet trials, each of which employed the "step-ramp" trajectory of Rashbass. The monkey initiated a trial by fixating the stationary target. At an unpredictable time, the target underwent a sudden horizontal displacement ("step") and began to move at 15 deg/s ("ramp") either towards or away from the fovea. After 500-1000 msec, the target was extinguished and the monkey received a water reward if he had tracked accurately throughout the trial.

For step amplitudes up to 20 deg, monkeys initiated pursuit while the image was still eccentric on the retina, and only subsequently made a saccade to bring the image onto the fovea. We estimated the "gain" of pursuit initiation as the eye velocity 100 msec after the initiation of pursuit divided by target velocity. Because the latency of pursuit is near 100 msec, our measurement represents the response to stimuli that occurred before the eyes began to move; we therefore knew the eccentricity and the velocity of the stimulus. The gain of pursuit initiation was highest for images that were close to the fovea and had maximum values of 0.39, 0.79 and 0.85 in the 3 monkeys. As image eccentricity increased, gain declined and was assymetric depending on the direction of image motion. At 5 deg eccentric, for example, gain was 77% of maximum for images moving towards the fovea, and 46% of maximum for images moving away from the fovea. The latency of pursuit was nearly independent of image eccentricity, but depended on whether images were moving towards (100-130 msec) or away from (65-90 msec) the fovea.

Our results imply that visual inputs to the pursuit system are most powerful near the fovea and become less powerful as eccentricity increases. In addition, our methods provide an objective means of assessing the defects caused by CNS lesions: measurements of pursuit initiation to moving stimuli at a series of retinal eccentricities will indicate whether pursuit has been affected only in one part of the visual field, or is deficient in a more general way related to the direction or velocity of the motor act.
Supported in part by grant EY03878 from the NEI.

81.2 EFFECTS OF BILATERAL OCCIPITAL LOBE LESIONS ON EYE MOVEMENTS IN PRIMATES: PRELIMINARY OBSERVATIONS, D. S. Zee, R. J. Tusa, L. M. Optican* and G. Gücer, Depts. of Neurology and Neurosurgery, Johns Hopkins Univ., Balto., Md. 21205 and National Eye Institute, Bethesda, Md. 20205.

Eye movements were recorded in three trained juvenile Macaque mulatta with bilateral occipital lobe lesions. After surgery two animals could orient to objects moving in the far periphery but showed no smooth tracking even two months post-op. A third animal appeared completely blind after surgery but 5 weeks later began to orient to and smoothly track large moving objects.

Optokinetic Responses were qualitatively similar in all three animals but the third showed the most complete and enduring deficits and its behavior will be discussed in detail. The major findings were a decreasing response to higher retinal slip velocities, loss or attenuation of the rapid, "pursuit" component of OKN and temporal-nasal predominance during monocular viewing. To constant velocity stimuli ($< 30^{o}$/s) eye velocity slowly rose (> 15s) to target velocity. Eye acceleration increased when retinal slip velocity decreased to 15^{o}/s. For a 60^{o}/s stimulus eye velocity only reached 8^{o}/s but with a 0.25^{o}/s^{2} accelerating stimulus, which keeps retinal slip velocity low, eye velocity reached 58^{o}/s. During monocular viewing, maximum velocity was 32^{o}/s for temporal-nasal and 10^{o}/s for nasal-temporal stimuli.

VOR Gain Adaptation: VOR gain (eye vel/head vel) in darkness was measured after 4 hours of combined chair and drum rotation (0.25 Hz, 30^{o}/s) with in phase (x0) or 180^{o} out of phase (x2) viewing. In the "cortically blind" monkey, during x0 viewing, the VOR gain dropped to 0.61 pre-op but only 0.74 post-op. During x2 viewing, VOR gain rose to 1.65 pre-op but only 1.13 post-op. After surgery, during x0 and x2 viewing, the VOR gains in the light and dark were the same.

Smooth Pursuit: After 8 weeks post-op, the "cortically blind" monkey recovered the ability to track a small, 0.25^{o} diam, moving target using both saccades and smooth pursuit. The latter could have gains (eye vel/targ vel) near 1.0. During monocular viewing pursuit was symmetric. Pursuit responses, though, were not sustained during OKN stimulation so the slow rise in eye velocity and temporal-nasal asymmetries persisted. This raises the issue: Are smooth pursuit and the rapid component of OKN identical?

Intermittent, predominantly horizontal, pendular oscillations (5 Hz, 1.0^{o} ampl) and inability to hold eccentric eye positions appeared post-op.

Our findings indicate that (1) primates have an underlying, perhaps subcortical, OKN system similar to that of afoveate animals, (2) VOR gain adaptation is impaired after occipital lobe lesions and (3) smooth pursuit can recover in a "cortically blind" monkey.

81.3 **RESPONSE PATTERNS OF VISUAL TRACKING NEURONS IN POSTERIOR PARIETAL CORTEX OF MONKEY DURING VISUAL-VESTIBULAR STIMULATION.** **Kenji Kawano*, Mitsuyoshi Sasaki* and Masayuki Yamashita* (SPON: F.A.Miles). Dept. of Neurophysiology, Inst. of Brain Res., Sch. of Med., Univ. of Tokyo, Tokyo, Japan.**

Visual tracking neurons in area 7a of the monkey are activated during smooth eye tracking in a particular preferred direction. Such neurons in the posterior part of area 7a continue to be active even when the moving target is extinguished for brief periods provided that tracking continues (as in darkness)[1]. This suggests that these neurons receive extra-retinal inputs during pursuit, and in the present study, we have examined further properties of these inputs, using visual-vestibular stimulation.

Monkeys were trained to fixate a target light, and were seated in a chair which could be oscillated about a vertical axis. The experiments were all performed in the dark except for the target light. We studied the discharge behavior of visual tracking neurons in four situations: 1) With the chair stationary, the monkey tracked a moving target with its eyes (pure eye tracking); 2) With the target and the chair moving together, the monkey had to cancel the vestibulo-ocular reflex in order to track the target (combined eye and head tracking); 3) The chair was oscillated in complete darkness, resulting in vestibulo-ocular responses with a gain 5-10% less than unity; 4) With the chair oscillating, the monkey fixated an earth-fixed target; since this target was relatively close (80cm), the associated translational movement of the eyes required that the VOR be supplemented 10-15% by tracking in order to maintain fixation (VOR target task).

The activity of more than 80% of the visual tracking neurons showed modulation during combined eye and head tracking which was similar to that during pure eye tracking, even showing similar non-linearities (e.g. saturation when velocities exceeded 15 deg/s). These data suggested that the activity of these neurons encodes gaze velocities. However, during oscillation in complete darkness, more than half of these neurons showed no modulation, despite the small but significant gaze velocity. Yet, during oscillation in the VOR target task when again gaze velocities were small, these neurons now showed some modulation (about 1/3 of that during pure eye tracking). These results suggest that these neurons only encode gaze velocity during tracking. The remaining visual tracking neurons that did show considerable modulation during chair oscillation in darkness may receive independent vestibular inputs in addition to the gaze signal.

1. Sakata, Shibutani & Kawano: Exp. Brain Res., 41: A27-A28, 1980.

81.4 VISUO-OCULOMOTOR INTERACTIONS IN DORSOLATERAL PONTINE NUCLEUS OF ALERT MONKEY. D.A. Suzuki and E.L. Keller (SPON: K. Nakayama). Smith-Kettlewell Inst. Visual Sciences, San Francisco, CA 94115.

The visual properties of dorsolateral pontine nucleus (DLPN) cells were studied in an alert monkey in order to shed some light on the visuo-motor transformations associated with smooth pursuit eye movement control. DLPN is a major terminus for tecto-, pretecto-, and cortico-pontine pathways, including afferents from the striate, prestriate, temporal (esp. Middle Temporal), and parietal cortices. Furthermore, DLPN projects to vermis-VI, VII and the flocculus, which are both cerebellar structures that figure prominently in oculomotor functions.

A macaque was trained to perform visuo-oculomotor tasks designed to dissociate retinal image, eye, and head movement-related signals. Retinal slip-related DLPN activity was recorded while the monkey suppressed its eye movements by fixating a stationary, reward-related spot during presentations of a moving random dot background pattern. Discharge rate was related either to (i) retinal slip velocity in a preferred direction or to (ii) only the direction of background movement. Some DLPN cells also exhibited pursuit eye movement-related activity when the monkey tracked a moving fixation spot. Differences were observed in DLPN responses to pursuit in the dark and in the presence of a background pattern suggesting visuo-oculomotor signal interactions.

When the background pattern was replaced with a small, non-reward-related 'test spot', some DLPN cells also exhibited responses to the moving, discrete visual stimulus. With the eyes stationary, two types of test spot-related, retinal slip responses were observed. (1) Some DLPN activity was sinusoidally modulated with sinusoidal test spot movement while (2) other DLPN cells exhibited visual responses characterized by two components: (2a) as the test spot crossed the fovea in a preferred direction, transient slip velocity-related increases in discharge rate occurred and (2b) a maintained, smaller increase in activity was observed for the duration of test spot movement in the preferred direction. Since the eyes were stationary during presentation of the visual stimuli, responses (1) and (2b) suggest large receptive fields. Furthermore, the two component visual response suggests the convergence of both large-field and small, foveally-centered receptive field signals onto some DLPN cells.

The responses to background movement were similar to mossy fiber responses in vermis-VI, VII and the flocculus. Vermis-VI, VII has been implicated in the control of both smooth pursuit and saccadic eye movements and is the only cerebellar structure wherein discrete, test spot induced retinal slip-related activity has been observed. The present results, plus anatomical reports, suggests that the DLPN plays an important role in the processing of visuo-oculomotor signals reaching the cerebellum.

81.5 INFLUENCE OF LONG-TERM OPTOKINETIC STIMULATION ON EYE MOVEMENTS OF THE RABBIT. N. H. Barmack and B. J. Nelson*. Physiology Section, The Biological Sciences Group, The Univ. of Connecticut, Storrs, CT 06268.

Optokinetic afternystagmus (OKAN) is the persistence and gradual decrease in velocity of eye movements when an animal is placed in the dark following optokinetic stimulation: usually rotation of a large contour-rich drum about the stationary rabbit. In rabbits, the slow phase of OKAN occurs in the same direction as the inducing optokinetic stimulus. This positive OKAN (or OKAN I) is usually followed by a variable reversal in slow phase eye movements (OKAN II). Positive OKAN lasts for tens of seconds to tens of minutes, reflects a temporary imbalance of visual-vestibular information, and is dependent upon an intact vestibular system. We have investigated the effects of prolonged optokinetic stimulation on spontaneous eye movements in the rabbit. Rabbits were placed in an optokinetic drum for periods of six hours-six days. Subsequently the rabbits were removed from the optokinetic drum and placed on a vestibular rate table where eye movements could be recorded and evoked by horizontal vestibular stimulation. Spontaneous eye movements in the dark were observed for a period of 15 minutes after which the HVOR was measured at frequencies of .02-.80 Hz. After optokinetic stimulation and between recording sessions, rabbits were maintained under two conditions: 1) Unrestrained in a dark enclosure, 2) Restrained under conditions in which visual, but not vestibular, stimulation was possible. Rabbits which received less than 12 hours of optokinetic stimulation had a positive OKAN which was proportional in velocity and duration to the previous optokinetic stimulation. Rabbits which received 1-6 days of optokinetic stimulation displayed a long lasting negative OKAN when placed in the dark: The slow phase of this negative OKAN attained velocities which were 5-20 times higher than the velocity of the optokinetic stimulus and exceeded the slow phase velocities which are recorded following hemi-labyrinthectomy. The development of this negative OKAN could be prevented by the pattern stimulation provided by the ambient lighting conditions of the laboratory. However, once the negative OKAN is developed visual feedback can no longer suppress it. If rabbits are restrained, but allowed visual stimulation following two days of optokinetic stimulation at 5 degrees/sec, the negative OKAN persists for 1-3 days. However, if rabbits are placed in the dark, unrestrained, the negative OKAN disappears within 6 hours. This long-lasting nystagmus reflects a profound change in the balance of the vestibular system. Experiments are in progress to determine at what level this imbalance occurs. (Supported by NIH grant EY04167).

81.6 PONTINE RETICULAR FORMATION LESIONS ABOLISH QUICK PHASES OF OPTOKINETIC HEAD NYSTAGMUS IN THE RAT. D.W. Sirkin, Y. Zedek*, and P. Teitelbaum. Program in Neural and Behav. Biol. and Dept. of Psychol., Univ. of Ill. at Urbana-Champaign, Champaign, IL 61820.

Lesions in the medial portion of the pontine reticular formation (PRF) abolish spontaneous lateral head movements in the open field and quick phases of vestibular head and eye nystagmus (Sirkin, D.W. et al., Exp. Neurol., 69:435, 1980). Since similar lesions in the monkey affected optokinetic as well as vestibular (ocular) nystagmus and saccades (e.g. Cohen, B. et al., Arch. Neurol. (Chic.), 18:78, 1968), we suspected that quick phases of optokinetic head nystagmus (OKHN) in the rat would be abolished by PRF lesions.

Unilateral or bilateral electrolytic lesions were made in the rat PRF as before. Rats were tested on a small pedestal inside an optokinetic drum. The movements of the drum and rat were recorded on video tape. Graphs of exemplary portions of the records were made by measuring angles on sequential still frames.

PRF lesions caused a lasting abolition of quick phases of OKHN. Rats with unilateral lesions circled to follow the moving stripes with their heads when the drum was rotated towards the intact side (i.e. clockwise for a rat with a left PRF lesion). When the drum was rotated towards the damaged side, the rats showed OKHN (quick phases in direction of intact side). Rats with bilateral lesions circled to follow the drum in either direction (no quick phases), and their eyes were similarly affected: there were slow phase deviations, but no resetting quick phases.

We also observed transient deficits in slow phases of OKHN, which may have been due to damage to visual pathways mediating optokinetic responses (Cazin, L. et al., Pflügers Arch., 384:19, 1980).

PRF lesions also abolished orienting head movements to a visual stimulus.

Thus, in the rat, the PRF is essential not only for eye movements, but also for a class of "fast" lateral head movements including spontaneous turns in the open field, orienting turns, and quick phases of nystagmus.

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81.7 FROG PREY ORIENTING: EVIDENCE FOR THE INVOLVEMENT OF AN UNCROSSED TECTOFUGAL PATHWAY. S.K. Kostyk and P. Grobstein. Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

Visual prey orienting in the frog is generally considered to depend on crossed descending tectal projections. We previously reported (Soc. Neurosci. Abstr., 6:75, 1980) that a transverse hemisection, cutting all pathways between midbrain and hindbrain on one side, abolishes turns toward stimuli at any location to one side of the midline while leaving turns toward stimuli on the other side unaffected. Binocular visual field (including space on both sides of the midline) is represented in both tectal lobes. Hence, the failure of hemisected frogs to turn to stimuli on one side of the midline implies that the unilateral lesion has affected outputs of both tectal lobes. This suggests the existence of an uncrossed tectofugal pathway involved in orienting behavior. Ingle, however, has reported (Analysis of Visual Behavior, MIT Press, 1982) that frogs with lesions cutting the crossed projections from both tectal lobes fail to show the spared orienting toward stimuli in binocular field, which would be expected on the assumption of uncrossed pathways. We here report new findings on the behavior of such frogs.

In six frogs we split the ventral midbrain so as to interrupt the crossing descending fibers from both tectal lobes. Consistent with the report of Ingle, none of these frogs turned toward stimuli located within binocular field, nor at more peripheral positions up to about 100°around the frog; they responded instead with forward movements. For still more caudal positions, however, the frogs did turn, with increasing amplitude for progressively more caudal positions. Turn amplitudes ranged from 0° to 100°. Turn directions were those predicted to be mediated by the uncrossed pathways; stimuli at positions mapped in the right or left tectal lobes caused rightward or leftward turns respectively. Appropriate stimuli provoked a combined turn and snap.

The results support the suggestion that there are uncrossed pathways from each tectal lobe adequate to produce orienting turns in the ipsilateral direction. Two facts may explain why the behavioral observations supporting this suggestion were previously overlooked. First, the behavior is not immediately evident in all frogs. Three animals showed the behavior described on first testing, within four days of the lesion. The remaining three initially failed to turn for stimuli at any location and first exhibited turning 3 to 14 days later. Second, the surviving turns were not activated by stimuli at the expected locations; they were revealed only by testing at more caudal positions. This dissociation of input and output direction may be important in analyzing how the brain is organized such that stimuli at given locations trigger the appropriate turns in normal frogs.

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81.8 CURRENT-DISTANCE RELATIONSHIP FOR NEURONS MEDIATING CIRCLING BEHAVIOR ELICITED BY TECTOPONTINE TRACT STIMULATION. J. S. Yeomans, P. Prior* and F. D. Bateman*. Dept. of Psychology, Univ. Toronto, Toronto, Ontario, M5S 1A1.

Fouriezos (1980) devised a behavioral method for measuring the distance (d) at which stimulating current (I) activates neurons mediating the observed behavior. The method involves placing two electrodes side by side at a known distance (near .5 mm), delivering paired pulses and measuring the degree of refractoriness. The present study makes three improvements on Fouriezos' results: 1) more reliable data are obtained by using circling induced by stimulation applied near the tectopontine tract (Miliaressis, 1981; Burne et al., 1981) instead of self-stimulation; 2) these more reliable paired pulse data allow separate measurement of the current-distance relationship in neural populations defined by refractory period; 3) a geometric model allows prediction of the shifts in refractoriness in uniform and non-uniform populations as a function of current.

For the relationship $I-I_0 = k\,d^2$, I_0 is less than 10 μA for electrodes with tips plus surrounding scars smaller than 100 μm in diameter. For neurons with refractory periods 0.4-0.6 msec, k is estimated to be 350 μA/mm²; for neurons with refractory periods 0.6-1.5 msec, k is estimated to be 900 μA/mm². The current thresholds of the short refractory period neurons, then, are on average less than half that of the longer refractory period neurons.

In addition, spatial summation between the two electrodes was observed to increase from 40 to 80% as current was increased to produce more overlap in the stimulating fields.

Burne, R. A., Azizi, S. A., Mihailoff, G.A. & Woodward, D. J. J. Comp. Neurol., 1981, 202, 287.
Fouriezos, G. Paper presented at Canadian Psychological Association, June, 1980.
Miliaressis, E. Physiol. Behav., 1981, 26, 709.

81.9 TECTOTECTAL INTERACTIONS IN THE CAT. A.K. Moschovakis* and A.B. Karabelas* (SPON: K. Suzuki). Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Intracellular recording and intracellular HRP staining were employed to physiologically identify commissural tectotectal neurons and study their synaptic action on neurons of the contralateral superior colliculus. Somadendritic morphology and axonal pattern of intracellularly HRP labeled tectal neurons were analyzed to disclose their possible classification into different cell types. Experiments were performed on barbiturate anesthetized cats.

Commissural tectotectal neurons were antidromically activated following stimulation of the contralateral superior colliculus with latencies of 0.9-2 msec. Intracellularly HRP labeled commissural neurons were predominantly located in the stratum griseum intermedium. Their fusiform cell body measured 350-625μ^2 in area and occasionally tended to assume a pear-shaped form. Dendritic trees were horizontally or vertically oriented. Only one multipolar (900μ^2) neuron with radiating dendrites was physiologically identified as commissural. Axons (2-3μ in diameter) of commissural neurons followed a ventral course and consistently collateralized. Either the main axon or 1-2 first order extrinsic collaterals were oriented towards the opposite superior colliculus and could bifurcate before or after crossing the midline. One to three intrinsic collaterals profusely arborized within the deep layers of the optic tectum. Non-intertectal axonal elements of commissural neurons assumed either crossed or uncrossed trajectories.

Contralateral collicular stimulation elicited monosynaptic IPSPs in tectal neurons with latencies of 1-2.4 msec. These IPSPs were usually preceeded by brief EPSPs with latencies of 0.4-1.5 msec and may originate, at least in part, from the activation of intertectal fibers. Intratectal (i.e. recurrent collaterals of tectotectal neurons) or extratectal (e.g. nigral) fibers may also participate in the generation of tectally evoked IPSPs. Intracellular HRP labeling revealed a variety of postsynaptic tectal neurons according to their location (stratum griseum intermedium or profundum), soma size (180-1600μ^2) and shape (multipolar, fusiform, polygonal, triangular), dendritic pattern (vertical, horizontal or isodendritic) and axonal pattern. Decussating axons 3.5-5.5μ in diameter) of large multipolar or fusiform neurons occasionally emitted one collateral at the level of the midbrain reticular formation. Axons (1-2.5μ) of small fusiform neurons emitted both intrinsic and extrinsic collaterals.

These data indicate that commissural tectotectal neurons form a class of tectal neurons with consistently collateralized axonal system and exert monosynaptic inhibitory action on a variety of neurons in the opposite superior colliculus.

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81.10 "EFFERENCE COPY" NEURONS IN CAT SUPERIOR COLLICULUS. Carol K. Peck. Department of Psychology, Pomona College, Claremont, CA. 91711.

Eye movement (EM)-related units in the superior colliculus (SC) of the cat fall into two groups which are distinguisable on the basis of the timing of their discharge: (1) those leading the onset of EMs by 50 ms. or more, and (2) those beginning to discharge less than 20 ms. prior to EMs. Units in the first group resemble units reported in the colliculus of rhesus monkeys, discharging prior to EMs of particular amplitudes and directions. In contrast, units in the second group are omni-directional, discharging prior to EMs of all directions and amplitudes. They resemble units reported in the optic tectum of fish and labeled as "efference copy" neurons.

Eye movements were recorded from both eyes of 6 cats via d.c. electrooculography (EOG) with chronically-implanted silver-silver chloride electrodes (Bond and Ho). Using water reinforcement, cats were trained to make EMs anticipating the appearance of a visual target at one of 4 locations (10° up, down, left or right of the center of a tangent screen located 114 cm. from the cat). All neurons included in this report were studied both in the presence of dim, red illumination of the screen and in total darkness. The behavioral task provided continuous calibration of the EOG signals. Units were recorded with Tungsten microelectrodes.

In 4 of 6 cats studied, "efference copy" neurons were encountered in the anterior half of SC within or immediately ventral to the stratum opticum. There did not seem to be any distinct medial-lateral organization, although I did not explore the most medial portions of SC in order to avoid damage to striate cortex. Wherever "efference copy" neurons were isolated, there was also unresolved background which was modulated with EMs of all directions and amplitudes.

These neurons were not responding to changes in visual input since they discharged with EMs in total darkness. Since they discharged with saccades in all directions, they cannot play a major role in the control of saccade direction. In addition, for a given cell, the timing of the discharge varied from saccade to saccade and, in some units, occasionally started after the onset of the EM. Therefore, it seems unlikely that they are causally involved in the initiation of saccades. Instead, they appear to signal the fact of an EM and to indicate roughly the time of its occurrence. Supported by NS-14116.

81.11 RESTRICTION OF HORIZONTAL EYE MOVEMENTS CAUSED BY LESIONS OF THE TECTUM AND PRE-TECTAL STRUCTURES. E.G. Keating, S.E. Pratt*, D.V. Kenney*, and S. Gooley*. Dept. of Anatomy, S.U.N.Y. Upstate Medical Center, Syracuse, N.Y. 13210.

Combined removal of the superior colliculus (SC) and frontal eye-fields (FEF) markedly restricts to about ± 10° the range of saccadic eye movements that a monkey can make in any direction from its position of primary gaze (Schiller et al., 1980). We report here a similar but selective narrowing in the range of horizontal eye movements produced by lesions which removed SC but also invaded mesodiencephalic structures just anterior to it.

Five monkeys (M. mulatta and fascicularis) learned to fixate a target, a 1° spot of light appearing at various points on a projection screen. They then received lesions intended to remove the SC on one or both sides. The surgery produced a subtle visual deficit: the monkeys had some difficulty finding the target but only when its contrast was reduced by embedding it in a background of visual distractors.

The more prominent deficit was an oculomotor one. When searching for targets or when retrieving apples from a board in front of them, the monkeys' eyes never wandered more than ± 8-15° in a horizontal direction from the position of primary gaze. The horizontal range of spontaneous movements was similarly reduced although a sudden noise could occasionally trigger a movement outside of this range. We tried unsuccessfully in one monkey to extend the range by directly rewarding more widely eccentric saccades. Vertical range in these 3 animals was normal or only slightly reduced. The deficit recovered in 3 weeks in one animal but persisted through the 2 and 4 months of observation in the other monkeys.

Although more severe in degree, the oculomotor deficit was alike in kind to that recently reported by Albano and Wurtz (1981). Our lesions and interpretation are similar to theirs. Tentatively it appears that the effect of combined SC and FEF lesions on visually guided horizontal eye movements can be mimicked by a lesion that removes most of SC and transects at the mesodiencephalic junction the dorsal bundle of efferents descending from the frontal eye fields (Leichnetz, 1981). In support of this interpretation, subsequent FEF removal in one of the affected monkeys caused no further restriction of horizontal range but shrank its vertical range to about ± 10° degrees. In another monkey which had recovered normal eye movements after tectal surgery, subsequent FEF removal reinstated the horizontal range deficit.

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81.12 ORGANIZATION OF SUBCORTICAL PROJECTIONS FROM SACCADIC EYE MOVEMENT SITES IN THE MACAQUE'S FRONTAL EYE FIELDS. G.B. Stanton, C. Bruce and M.E. Goldberg. Dept. Anatomy, Howard Univ. Coll. of Med., Washington, DC 20059, Lab. Sensorimotor Res. National Eye Institute, Bethesda, MD 20205, and Dept. of Neurology, Georgetown Univ., Washington, D.C. 20007.

The frontal eye fields (FEF) of the monkey cerebral cortex lie in the most caudal part of the prearcuate gyrus, just anterior to the bend of the arcuate sulcus. This region contains neurons that discharge prior to purposive saccadic eye movements, and microstimulation at low currents (10 - 50 μa) elicits saccades. The representation of eye movements is organized so that larger movements are located dorsomedially and smaller movements ventrolaterally in the FEF. This low threshold FEF, which is largely buried in the rostral bank of the arcuate sulcus, is considerably smaller than previous physiological and architectonic studies have indicated.

In six macaques the low threshold FEF were located by electrical stimulation and then injected with tritiated amino acids (proline/leucine). Subcortical labeling was most dense and abundant in the rostral thalamus. Two large terminal fields were found lateral (VAmc, VLx, dorsomedial VLc) and medial (MDpc) to the internal medullary lamina, but no terminal labeling appeared in the intralaminar Pcn nucleus. Terminal labeling in the caudal thalamus consisted of small patches in MDpc and Pf and sparse amounts of label were seen in the medial pulvinar and the nucleus limitans. Terminal patches found in the subthalamus and rostral midbrain tegmentum were smaller and less dense than those in rostral thalamus. Label was found in the ventrocaudal subthalamic nucleus, dorsomedial part of the parvocellular red nucleus, and the nucleus of Darkschewitsch. Light labeling was seen in the nucleus reticularis tegmenti pontis (bilaterally) and in the parabigeminal nucleus. Discrete, dense patches of label were found in the dorsolateral and medial pontine nuclei. There was no label in the oculomotor or preoculomotor nuclei.

Each case projected densely to the intermediate layers of the superior colliculus (SC). Only the most dorsomedial injection had label in the deep layers and only the most ventrolateral case had light labeling in the superficial layers. The projection to the intermediate layers was topologically organized. Thus the dorsomedial region of the FEF, where the largest saccades were elicited, projected exclusively to the caudal 2/3 of the SC, in which large saccades are represented. Only injections in the ventrolateral region of the FEF, where the smallest saccades were elicited, projected to the rostrolateral SC, which represents small saccades. Supported in part by NIH 1 RO1 EY 03763-01.

81.13 VISUAL PATHWAYS FROM THE SUPERFICIAL SUPERIOR COLLICULUS TO THE BASAL GANGLIA C.-S. Lin and W. C. Hall, Department of Anatomy, Duke University, Durham, North Carolina 27710

Substantia nigra pars reticulata (SNR) receives projections from the corpus striatum and projects to the intermediate grey layer of the superior colliculus. In the monkey (Wurtz and Hikosaka, Hikosaka and Wurtz, Neurosci. Abstr., 7: 132, 1981), many nigrotectal neurons respond in relation to saccadic eye movements to targets in limited parts of the visual field. We present evidence here that visual information may reach SNR from the superficial grey layer of the superior colliculus in the tree shrew (Tupaia glis) and grey squirrel (Sciurus carolinensis). From previous studies, we know that the large cells in the lower half of stratum griseum superficiale project to the pulvinar. In the present experiments, small electrolytic lesions (10-50 μamps) or injections of tritiated amino acids (.05-0.5μl,20μCi/μl) were made in the pulvinar for the purposes of tracing anterograde axonal degeneration or transport, respectively. In both species, prominent, topographically organized projections could be traced to the caudal half of the corpus striatum. Since the corpus striatum projects to SNR, this particular pathway may provide an important source for visual control of the eye-movement related responses characteristic of the nigral neurons which project to the intermediate grey layer of the superior colliculus. (Supported by EY04060 and NSF BNS-8109794 to W. C. Hall and NS-17619 to C.-S. Lin).

81.14 CONVERGENCE OF ALPHA RHYTHM AND FINGER TREMOR FREQUENCIES INDUCED BY RHYTHMICAL PHOTIC STIMULATION. M. Isokawa and B.R. Komisaruk. Institute of Animal Behavior, Rutgers Univ., Newark, NJ 07102.

There is an overlap in the range of frequencies of the EEG alpha rhythm (8-12 c/sec: Spehlmann, EEG Primer, 1981) and physiological finger tremor (8-12 c/sec: Lippold, J. Physiol., 206:359, 1970; 5-15 c/sec at a peak amplitude of 9-10 c/sec: Halliday and Redfearn, J. Physiol., 134:600, 1956). This raises the question of whether these two rhythms are independent of each other or whether they are influenced by a common mechanism, i.e. "coupled." For the purpose of analyzing the relationship between alpha rhythm and finger tremor, we recorded EEG differentially between occipital and prefrontal scalp areas in humans, using silver-silver chloride pregelled electrodes, and finger tremor by a single plane accelerometer from an index finger. Two different conditions, with or without photic stimulation were analyzed, in order to determine the degree of coherence corresponding to each condition by analyzing their frequency distribution. Data for analysis were selected by accepting only alpha wave epochs which showed a regular shape and relatively high amplitude (100-150 μv) with finger tremor of 6-13 c/sec, persisting for more than 1 sec in the recording periods.

Rhythmical tremor of 9.7 c/sec ± 1.1 (group mean ± SD; individual mean range: 8.3 ± 1.1 to 11.4 ± 1.8) was accompanied by a stable alpha rhythm of 10.3 c/sec ± 0.5 (individual mean range: 9.2 ± 1.1 to 11.0 ± 1.1) spontaneously, showing a one-to-one relationship at times. When photic stimulation was provided at a frequency close to the subject's spontaneous alpha rhythm, frequency-convergence occurred between alpha rhythm (10.6 c/sec ± 0.4) and finger tremor (10.3 c/sec ± 0.1). The correlation coefficient between two rhythms under photic stimulation was significantly higher (0.44 ± 0.14 SD, $P < .05$) than under the spontaneous condition (0.13 ± 0.16). The comparison of the mean frequency-deviation of finger tremor from alpha rhythm showed an effect of photic stimulation on increasing the coherence significantly ($\bar{X} = 1.23 \pm 0.82$: spontaneous; $\bar{X} = 0.83 \pm 0.46$: photic stimulation; $P < .05$, $t = 2.54$, t-test for correlated means). Our findings indicate that alpha wave and finger tremor tend toward synchrony with each other in an environment of flickering photic stimulation, suggesting that the two systems may normally be functionally related, and not independent of each other. Supported in part by NSF Grant BNS 78-24504 (BRK).

81.15 VISUALLY TRIGGERED OPEN LOOP REACHING FOR A MOVING TARGET AFTER DORSAL RHIZOTOMY. T.A. Tran, D.E. Teodoru, and A.J. Berman. Dept. of Neurosurgery, VA Medical Center, Bronx, New York 10468.

In an earlier report (Teodoru, et al, 1979) we attributed the ataxia when reaching with a dorsal rhizotomized (DR) forelimb to the attempted use of visual guidance. Lacking predictive velocity input from muscle receptors such closed loop movements exhibit the destabilizing effect of the long visuomotor feedback loop delay (Murphy, et al, 1975), hence the ataxia. However, as the DR monkeys learned to use visual input before rather than during movements to program a reaching trajectory, the movement became smooth and accurate. This study sought to test whether visual input could be used in the same way by monkeys subjected to right sided DR (C_2-T_3) and required to reach for a piece of food afixed to the periphery of a rotating turntable mounted vertically with its axis of rotation perpendicular to the monkey's body. The lower half of the turntable was covered by a partition, making the food visible and accessible through an arc of 180°. The turntable rotated at 96, 198, 270 or 468°/sec., randomly varied from trial to trial. On each trial the turntable was allowed to rotate until the responding hand made contact with it. At 96, 198 and 270°/sec., the reaching response of the DR limb was smooth, accurate and rapid, always meeting the target in the same 100 to 120° zone. The greater the speed of the turntable the greater the latency to response onset (i.e. the greater the number of rotations). The monkeys kept their heads stationery, facing forward and the eyes followed the target in smooth pursuit. When the target moved at 468°/sec., however, the monkeys exhibited head movements and discontinuous, jerkey, eye movements in apparent attempts to follow the target. At this speed, reaching with the DR limb was never successful, exhibiting wide amplitude lateral oscillations (2-5 Hz) of proximal origin.

We conclude that the reaching movement was performed in open loop fashion, triggered by predictive computations of target position based on estimates of its velocity from visual pursuit. However, when the speed of the target was too great for smooth pursuit, the monkeys attempted to follow the target with saccades; saccade input does not provide for adequate velocity readout, so the monkeys apparently returned to visual guidance, causing reaching ataxia.

82.1 CONTRAST SENSITIVITY CHANGES IN AN INFANT MONKEY WITH EXTENSIVE TRANSSYNAPTIC GANGLION CELL LOSS FOLLOWING STRIATE CORTEX LESIONS. J. Dineen*, B. Vermeire* and R. Boothe. Dept. of Ophthalmol. RJ-10, Univ. of Wash., Seattle, WA 98195, and Div. of Biol. 156-29, Calif. Inst. Tech., Pasadena, CA 91225.

Adult Macaca monkeys can demonstrate a number of complex visual capacities after the complete loss of striate cortex (Dineen & Keating, '81). Anatomical evaluation of these behaviorally tested, adult-lesioned animals revealed that even though massive retrograde degeneration of the dorsal lateral geniculate nucleus (dLGN) was present, transsynaptic retrograde degeneration of retinal ganglion cells was small (Dineen et al., '82). This result is in marked contrast to monkeys that are lesioned during infancy where we have shown that retinal ganglion cell loss is very large (Dineen & Hendrickson, '81). To assess whether visual capacities are more severely restricted in infant-lesioned monkeys who show massive ganglion cell loss accompanying striate cortex lesions, we have behaviorally tested a monkey which had undergone bilateral removal of striate cortex during the first postnatal year.

Behavioral testing consisted of psychophysically measuring the contrast sensitivity functions (C.S.F.) for gratings of varying orientations using an automated operant testing apparatus. After preoperative testing, our Macaca nemestrina monkey received bilateral removal of the central 20° of striate cortex at 12 months of age. At the completion of postoperative testing, anatomical examination of the extent of the cortical lesions confirmed that the central 20° of striate cortex was removed. The posterior two thirds of both dLGNs showed a nearly complete loss of all neurons and the central 20° of retina had undergone approximately an 80% loss of ganglion cells.

Our psychophysical testing revealed three main results. First, there was a loss of a preoperative oblique effect after cortical lesions, indicating that central striate cortex may play a significant role in orientation bias. Secondly, there was improvement in the C.S.F. to near normal preoperative levels for low spatial frequencies. Thirdly, an overall acuity value was estimated at 15 cy/deg., a level 3 times greater than acuity measurements in normal humans at 20° eccentricity.

The results from this study demonstrate that even though infant monkeys show a far greater loss of retinal ganglion cells after striate cortex damage than adult-lesioned monkeys, their visual capacity remains remarkably high. Supported by Grants EY-07013, EY-03956 and EY-01208.

82.2 Critical Period for a Corpus Callosum Role in Visual Acuity in Cat is Restricted to the First Postnatal Month. Andrea J. Elberger. Dept. of Neurobiology and Anatomy, University of Texas Medical School at Houston, Houston, Texas 77025.

It has previously been reported that neonatal corpus callosum section in cats produces permanent deficits in visual acuity (Elberger, Soc. Neurosci. Abstr. 6: #171.9, 1980). This result has been extended to determine age limits for this phenomenon.

Three groups of neonatal callosal sectioned cats were tested: surgery at 1-7 days (3 cats), at 15 days (6 cats) and at 21-22 days old (7 cats). Visual acuity was measured by determining the detection threshold of 95% contrast square wave gratings on a modified jumping stand; a 70% correct criterion was used. Threshold testing occurred binocularly and monocularly each week from 6 to 23-45 weeks of age. The data from the three groups of neonatal callosum sectioned cats were compared with data from 8 controls.

All 4 groups showed a gradual improvement in acuity throughout the period of testing. In general, from 14-18 and from 24-29 weeks of age, cats in all groups tended to display greater changes in visual acuity thresholds than at other times. However, the acuity thresholds differed in all 4 groups. There was a significant negative correlation between age at surgery and extent of acuity deficit such that the final acuities of the 1, 2 and 3 week sectioned cats were arranged in a linearly increasing distribution. The acuity deficits for the 1 and 2 week callosectomized cats were statistically significant. However, while the mean acuity for the 3 week sectioned cats was lower than that of the controls, the difference was not significant.

A previous study indicated that when callosal section occurs at 13-18 days of age there is a significant negative correlation between age at surgery and alteration of ocular dominance in Area 17 (Elberger, Exp. Brain Res. 41:280, 1981). This also suggests that there is a critical period of the first postnatal month for developmental effects of callosectomy. The acuity deficits may be a result of the interruption of one eye's input to a specific population of binocularly activated cells (e.g. disparity detectors) or may be a function of the numerical decrease of binocularly activated cells. Neither hypothesis is clearly supported and both may be responsible for the observed results. In a normal cat experimental manipulations begun after the first month result in acuity deficits; since this occurs when corpus callosum section is no longer effective in producing acuity deficits this suggests that different mechanisms produce the deficits in each case. The critical period for callosal alteration of the development of visual acuity precedes the most sensitive period of development of the visual cortex, which the callosum interconnects. This indicates that there may be several major periods of plasticity for the developing visual system. Supported by NIMH Grant 1 R03 MH36526-01.

82.3 RECEPTIVE FIELD PROPERTIES OF TRANSCALLOSAL NEURONS IN THE VISUAL CORTEX OF NORMAL AND REELER MICE. Peter A. Simmons and Alan L. Pearlman, Depts. of Physiology and Biophysics and Neurology and Neurological Surgery, Washington University Medical School, St. Louis, Missouri 63110

To continue our investigation of the role of laminar position in the development of the connections of cortical neurons, we have examined the receptive field properties of neurons in the visual cortex of normal and reeler (rl) mice that project to the contralateral cortex. We chose this population for analysis in order to determine whether the connections underlying specific receptive field properties such as orientation tuning and directional selectivity are altered by the developmental abnormality in neuronal position that characterizes reeler neocortex.

Transcallosal neurons near the border between areas 17 and 18a were identified by antidromic stimulation delivered through bipolar electrodes in the contralateral cortex. A computer controlled the visual stimuli, data acquisition, and analysis. Callosal neurons were distributed throughout the depth of the cortex in both normal and reeler and had similar latencies to antidromic stimulation.

There was no significant difference between normal and reeler callosal neurons in the distribution of their responses to moving stimuli of different speeds, orientations, or directions. In both phenotypes, most cells responded to a wide range of stimulus velocities with very little velocity tuning. Orientation tuning varied within both normal and reeler populations; we encountered similar frequencies of non-oriented, broadly-tuned, and sharply-tuned units in both. Directional selectivity also occurred with equal frequency in normal and reeler receptive fields. Some indication of differences between normal and reeler receptive fields was evident in their responses to stationary stimuli; more normal units exhibited an inhibitory surround as demonstrated by a diminished response to a large as compared to a small spot.

Our results indicate that the pattern of synaptic connections responsible for orientation tuning and directional selectivity in the receptive fields of transcallosal neurons in reeler are not significantly different from those in the normal mouse. This finding suggests that the laminar position of neurons in the neocortex is not a major determinant in the formation of cortical interneuronal connections. (Supported by NIH grants R01-EY00621 and T01-EY00092 from the National Eye Institute.)

82.4 ALTERATIONS OF VISUAL CORTEX DEVELOPMENT FOLLOWING INTRAOCULAR INJECTION OF AN INHIBITOR OF AXOPLASMIC TRANSPORT. M.A. Matthews Dept. Anat. LSU Med. Ctr. New Orleans, La. 70119

Graded doses of colchicine (10^{-5}—2 X 10^{-2}M) were injected into the eye of albino rats at 1,3,5,10,15 and 20 days postnatal to produce a partial suppression of axoplasmic transport during the critical phase of development of the visual pathway. Our previous studies demonstrated a reduction of up to 70% in the amount of ^{3}H Proline transported from the eye to the dorsal lateral geniculate nucleus (DLGn) and tectum, and in the amount of isotope transneuronally transported to the cortex. Examination of the retina revealed that intraocular colchicine severely retarded the development of sensory cell processes but all other neurons, particularly ganglion cells, were preserved (Neuroscience 7:365-384, 385-404; 1982). Correlative rapid Golgi and EM analysis of the DLGn showed a reduction in total nuclear volume, dendritic stunting and a diminution in the size and complexity of the synaptic glomerulus yet mean numbers of neurons in the nucleus remained stable, (Neuroscience 7:405-422; 1982).

The developing visual cortex was analyzed at various intervals following inhibition of axonal transport by employing quantitative Golgi and EM methodology. This study revealed a slight reduction in the thickness of the cortical plate, occurring principally in Layer IV. Concomitantly, the apical dendrites of Layer V pyramids demonstrated a small but significant reduction in the numbers of spines. A Zeiss MOP-3 image analyzer was used to measure the total numbers of synapses within constant defined areas of the cortex together with the area of individual synaptic profiles. The number of profiles, was reduced from 320±14/370μm^2 in controls to 188±47/370μm^2 in those animals receiving 10^{-3} — $2x10^{-2}$M colchicine in the contralateral eye. Further, the average area of cortical synapses increased from 0.207±S.E. .073μm^2 in controls to 0.325±S.E. .0161μm^2 in colchicine-injected animals and this effect was most profound in animals injected at birth through 5 days. Finally, histochemical analysis of cortical cytochrome oxidase and succinic dehydrogenase revealed a depression in the activity of these enzymes in colchicine-treated animals.

These findings indicate that pharmacologically-induced suppression of axonal transport within optic fibers produces a controlled deprivation during cortical development and can be used to further examine subtle mechanisms of transneuronal stimulation associated with maturation of visual cortical neurons and neuropil.

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82.5 ALTERATIONS OF CALLOSAL CONNECTIONS FOLLOWING EARLY STRABISMUS. N. Berman and B. R. Payne. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

The corpus callosum of the cat achieves its adult pattern of connectivity postnatally. At birth the region containing cells which project through the corpus callosum (callosal cell zone) is widespread in areas 17 and 18, and it contracts during the first few postnatal months. The region containing callosal terminals (callosal terminal zone), in contrast, attains its adult form by the end of the first postnatal week (Innocenti '81). Lund et al., ('78) and Innocenti and Frost ('79) have reported that the final distribution of callosal cells and terminals depends on the animal's visual experience. They found that in cats whose visual axes have been misaligned by artificial strabismus early in life, the callosal connections were more widespread than normal. Their results were consistent with the idea that callosal connections change postnatally to link the representations of the midline of the visual field in each hemisphere. Implicit in this hypothesis are certain situations in which expanded zones should and should not be observed. For example, in animals with unilateral convergent strabismus, widespread callosal cell and terminal zones would be expected only in the hemisphere contralateral to the strabismic eye. To test this hypothesis, we reared kittens with either medial or lateral misalignment of the right eye surgically induced at different ages. We then studied the location and extent of the callosal cell and terminal zones in adulthood. For each animal, the eye position was determined accurately under paralysis. The callosal cell zone was delimited by using retrograde transport of horseradish peroxidase. The extent of the callosal terminal zone was determined by anterograde transport of isotope. In all of the strabismic cats a greater proportion of area 17 than normal contained callosal cells. This occurred regardless of the size or direction of the strabismus and regardless of which hemisphere was examined. In addition, in each of the hemispheres containing an expanded cell zone, an expanded terminal zone was also present. The fact that the expanded zones were observed in both hemispheres argues against the proposal that the alterations in callosal connectivity provide appropriate connections between midline representations of the two hemispheres. A more parsimonius interpretation is that abnormal visual experience leads to an arrest of development of the callosal cell zone. The abnormal callosal terminal zone may then occur when axons of the abnormal callosal cells grow into territory previously unoccupied by axons from the contralateral hemisphere.

82.6 A THEORETICAL FRAMEWORK ENCOMPASSING GENERALIZING AND DISCRIMINATING UNITS APPLIED TO FEATURE SENSITIVE NEURONS IN VISUAL CORTEX. P. W. Munro*, C. L. Scofield* and L. N Cooper. Center for Neural Science, Brown University, Providence RI 02912.

A theoretical framework has been devised to describe the complimentary functions of generalization and discrimination by neurons. According to this model, a neuron evolves such that it either responds preferentially to one stimulus of its pattern environment, or responds most highly to that component common to all of those inputs. Both cases are described by a single first order differential equation for synaptic modification. The rate of change of synaptic efficacy is expressed as a product of the presynaptic activity and a nonlinear modulatory function of two postsynaptic variables, namely the spatially integrated activity of the cell and a threshold variable which is determined by a temporal integration of that activity. Whether the cell evolves to seek maximum (discrimination) or minimum (generalization) selectivity depends on fixed parameters included in the postsynaptic modulatory function. Generalizing neurons modify their afferent synapses so that they "encourage" patterns yielding responses below the modification threshold and "discourage" stimuli yielding higher responses. Discriminating cells operate in the opposite fashion. A pattern is encouraged (discouraged) by modifying the synapses so that they would produce a higher (lower) response if the same pattern were immediately presented.

A network incorporating both types of cells has been designed to model the feature sensitive properties of neurons in area 17. Cortical neurons seeking maximum selectivity rely on inhibition to suppress their response to non-preferred stimuli [1]. Inhibitory generalizing cells serve to suppress the component common to a pattern class and thus help the cell "separate" the patterns. This application is an extension of the previous work [2] which utilized the ideal synapse [3], which could functionally shift its mode between excitatory and inhibitory. Partitioning the cells into two classes resolves this idealization into three synapse types: G-I, G-D, and I-D (G = geniculocortical afferent, I = inhibitory generalizing cortical neuron, D = discriminating cortical neuron). To achieve maximum selectivity, the net inhibition to a cell must become strong enough to offset whatever excitation (there is bound to be some) is produced by the non-preferred patterns.

(Supported by ONR grant N00014-81-K-0136)

[1] Sillito, A.M. (1975) J. Physiol. (Lond.) 250:305-329
[2] Nass, M.M. and Cooper, L. N (1975) Biol. Cybern. 19:1-18
[3] Bienenstock, E.I. et al. (1982) J. Neurosci. 2:32-48

82.7 DEVELOPMENT AND MECHANISMS OF VISUAL CORTICAL SPECIFICITY, A.B. Saul* (SPON: P.B. Brown). Dept. of Physiol., West Virginia U., Morgantown, WV 26506.

We model the mechanisms by which cells come to respond only to a narrow range of visual stimuli, such as to a vertical bar but not to a bar slanted 15° off vertical. Inhibitory influences have been shown to be important in tuning cells (A.M. Sillito, J Physiol. 250:305-329, 1975). This model introduces a Hebb-type modification of inhibitory synapses which matches the modification generally assumed for excitatory synapses.

The retinocortical pathway is simplified to a direct excitatory mapping of the visual field onto a layer of cortical cells which are interconnected by a net of inhibitory synapses. Both the excitatory retinocortical connections and the inhibitory intracortical connections obey a rule which modifies their strengths by weakening inactive and strengthening active synapses. An abstracted visual environment is input in the form of gaussian curves along a single dimension, corresponding for example to seeing an oriented bar.

The system develops specificity using a wide range of parameters (speed of modifications, threshold, numbers of cells, initial synaptic strengths, input characteristics, and various system perturbations such as addition of excitatory intracortical neighborhood interactions). Using the measure of selectivity defined by Bienenstock et al. (J Neurosci. 2 #1:32-48, 1982) specificity rises typically from the order of .1 to about .7 on a scale from 0 (nonselective, a flat tuning curve) to 1 (selective, a delta-function tuning curve). Along with the development of this selectivity, the responses of cells become stable in the long term because of their sharp tuning and the resultant inability of inappropriate stimuli to modify their synapses. The ratio of the speed of inhibitory synaptic modification to the speed of excitatory modification appears to be crucial to this stability.

The assumption that all of the synapses in this system are modified by neural activity in the same way leads to cortical cells being inhibited by cells with similar response properties, rather than opposing preferences as is sometimes assumed. Simultaneous activity will increase the mutual inhibition between cortical cells. This means that the inhibitory influence onto a cell grows as development proceeds. This influence narrows the tuning curve beyond what would be expected due to the tuned afference, and predicts observed effects such as inhibitory flanks, as a result of the superposition of the excitatory tuning curve of a cell with the inhibitory input of cells with overlapping responses.

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82.8 UNEQUAL ALTERNATING MONOCULAR DEPRIVATION DOES NOT PRODUCE UNEQUAL VISUAL ACUITY. T.M. Reeves, R.N. Holdefer & D.C. Smith. Developmental Biopsychology Lab, Southern Illinois University, Carbondale, IL. 62901

In cats reared with unequal alternating monocular deprivation (AMD, 8-hrs vs 1-hr), 79% of striate cortical cells are driven by the 8-hr eye, and only 16% are driven by the 1-hr eye (Tieman et al., Neurosci. Abst., 1979, 5, 810.). Furthermore, cells driven through either eye display normal orientation tuning and direction selectivity. We have obtained measures of visual acuity through each eye of AMD-8/1 cats, to determine if acuity is correlated more with the number of cells driven by an eye, or with the response specificity of cells driven by the eye. Following the collection of acuity data, the cats were anesthetized and subjected to acute extracellular unit recordings, in order to confirm the shift in ocular dominance that is associated with AMD.

Kittens were dark-reared for the first 5 wks of life, then subjected to a regimen of AMD by giving one eye 8 hrs of experience on one day, and giving the other eye 1 hr of experience on the following day, and so on. A large opaque contact lens was used to cover the eye not receiving experience. Throughout their development (after 5 wks), kittens were trained in the jumping stand apparatus used to measure acuity.

After 9 months of AMD, final acuity estimates were obtained for three AMD-8/1 cats, and for two control cats (AMD-8/8 and AMD-1/1). Visual acuity thresholds did not differ between either eye of the AMD-8/1 cats. This suggests that acuity, for cats reared under the present conditions, is related more to the specificity of cells in visual cortex than to the number of responsive cells per se.

Cells were recorded in the medial bank of the lateral gyrus using conventional single-unit techniques and an unbiased sampling procedure. In close agreement with Tieman et al. (1979), we found 84% of cells to be responsive to the 8-hr eye, and 17% to respond to the 1-hr eye. Cells driven through either eye did not differ with respect to the response properties of orientation tuning and direction selectivity. The mean range of orientation tuning was ±31.0 degrees for the 1-hr eye and ± 33.7 degrees for the 8-hr eye.

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82.9 THE EFFECTS OF RESTRICTED VISUAL EXPERIENCE ON THE DEVELOPMENT OF OCULAR DOMINANCE PATCHES IN THE CAT. N.V. Swindale* (SPON:J.D.Bousfield). The Physiological Laboratory, Downing St., Cambridge CB2 3EG, U.K.

In normal 2 week old kittens, trans-neuronal autoradiography following injection of one eye with labelled amino acids reveals a uniform distribution of geniculate inputs in layer IV of area 17. Given normal visual experience these inputs gradually segregate over the next few weeks to form the adult pattern of ocular dominance patches (LeVay et al.,JCN,179,223,1978). If visual experience is absent (Swindale,Nature,290,332,1981) or if retinal activity is silenced with TTX (Stryker,Soc. Neurosci. Abstr.,7,842,1981) normal segregation is prevented.

At the meeting I will report further results showing that:
1) dark rearing does not prevent the formation of ocular dominance columns in area 18;
2) binocular lid suture is as effective as dark rearing in preventing normal segregation;
3) short periods of visual experience each day, spread out over a period of 4 months, will allow segregation to occur. As the total amount of experience given (about 170 hours) was roughly equal to that which a normal kitten would have had by the time segregation was complete, this suggests that the long periods in the dark did not reverse the segregation process. Periods of visual experience, however distributed in time, may thus sum linearly in promoting column formation.
4) Preliminary results show that exposure to stationary sine wave gratings of 0.25 or 0.5 cycles/deg, in goggles worn for similar short periods each day, allowed at most only incomplete segregation to occur. Complete segregation may thus require other spatial frequencies than were used in the experiments, movement, or some more complex attribute.

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82.10 DARK REARING ALTERS PATTERNS OF METABOLIC ACTIVITY. A.W. Toga and D.A. Stein*, Dept. of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Monocular enucleation of neonates results in electrophysiologic changes in the visual system of many animals. In the rat, anatomic reorganization is also observed in the form of axonal sprouting or enlargement of uncrossed optic pathways. Similar abberations may be induced by significantly altering the visual environment during development. The experiments reported here measured the metabolic rates of optic centers of rats raised in one of two visual conditions. Pigmented rats were reared from birth, with both eyes intact, in either ambient laboratory light or total darkness. All animals were allowed to mature for at least ninety days prior to the experiment. On the day of the experiment, rats were implanted with femoral arterial and venous lines and prepared for quantitative autoradiography using the ^{14}C-2-deoxyglucose (DG) method. During the surgery for cannulae insertion, one eye was removed. Comparisons of cerebral glucose utilization could therefore be made for intact versus deafferented optic centers. Glucose utilization was measured in ambient laboratory light for both dark and ambient reared rats.

Removal of one eye on the day of the experiment in ambient reared rats depressed the metabolic rates of contralateral optic structures. Glucose utilization in the superior colliculus (S.C.) was .81 and .58 µmoles/gm/min for the intact versus the deafferented side, .82 versus .62 for the dorsal lateral geniculate (LGNd) and .98 versus .88 for area 17 of the cortex. Throughout the visual system the intact side had higher metabolic rates than the deafferented side with differences of 40% for S.C., 32% for LGNd and 11% for area 17. Subjects that were raised in total darkness demonstrated more severe intact versus deafferented differences in glucose utilization. The rates of glucose utilization were 1.51 versus .58 µmoles/gm/min. for S.C., 2.02 versus 1.09 for LGNd and 1.16 versus .80 for area 17 of cortex. These values represent 160%, 85% and 45% differences for the S.C., LGNd and area 17 respectively. The large difference between intact and deafferented structures in dark reared rats exposed to ambient illumination was primarily the result of a marked increase in metabolism of the intact structures. These experiments demonstrate that patterns of metabolic activity can be influenced by different rearing conditions imposed on a developing animal.

82.11 MONOCULAR SUTURE DISRUPTS THE FUNCTIONAL ORGANIZATION OF RABBIT STRIATE CORTEX. E. Hazel Murphy. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Previous studies have reported that, in rabbits, early lid suture delays but does not prevent the attainment of normal, mature function in single neurons of primary visual cortex. However, these studies used the percentage of receptive field types encountered as their major index of normal function. We have re-examined the effects of early lid suture using quantitative assessments of receptive field characteristics of single neurons in the monocular region of primary visual cortex.

One eyelid was sutured prior to the time of natural eye opening and recordings were made when the animals were 2-4 months of age. In orientation selective cells, we measured the preferred orientation, the selectivity of orientation tuning, direction selectivity, and receptive field size. In non-orientation selective cells, we measured velocity sensitivity, preference for the onset or offset of light, and the influence of internal inhibition and of surround inhibition.

In orientation selective cells, two major consequences of deprivation were observed. There was a significant loss of direction selectivity with only 58% of orientation selective cells showing direction selectivity in deprived cortex, compared with 90% in normals. In addition, the mean receptive field size was 50% larger in deprived cortex, compared with normals. There were no significant changes in orientation preference or orientation tuning. Overall, the percentage of orientation selective cells was not changed. However, in normal animals, approximately equal numbers of Simple I cells (which have only 1 response area) and of Simple II cells (which have 2 or more antagonistic response areas) were encountered. In contrast, in deprived animals, twice as many Simple I as Simple II cells were encountered.

All of these changes, observed in orientation selective cells, can be interpreted as reflecting loss of inhibitory processes in deprived cortex. However, this effect was selective for orientation selective cells. Non-orientation selective cells showed no change in internal or surround inhibition or in velocity selectivity. These data indicate that, even in a mammal in which the visual pathways are predominantly crossed and in which binocular competition is therefore not a major factor, early deprivation of patterned visual experience results in significant changes in functional organization of visual cortex. These changes are selectively observed in orientation selective cells and may reflect an alteration in inhibitory processes.

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82.12 THE EFFECTS OF UNILATERAL INTERRUPTION OF DIRECT VISUAL INPUT IN ADULT CATS REARED WITH PREVIOUS MONOCULAR OR BINOCULAR VISION. M. Podell* and U. Yinon* (SPON : Y. LASS). Physiological Lab., Goldschleger Eye Inst., Tel Aviv Univ. School of Med., Sheba Med. Center, Tel Hashomer 52621, ISRAEL.

The effects of isolation of one hemisphere from direct visual input via unilateral optic tract section (OTX) were studied on nine adult cats, four of which had been monocularly deprived during the critical development period. Eleven animals served as respective controls. Extracellular recordings from areas 17, 18 and their border were performed at various intervals after OTX.

Results from the isolated hemispheres (n=226) of both OTX groups revealed that contralateral input via the corpus callosum was virtually inactivated. While the vast majority of the cells encountered were spontaneously active yet unresponsive, a small number of cells were initially suspected of exhibiting activity, but could not be confirmed as such by our criteria. Only four cells (3.5%), all from the deprived OTX cats, showed visual activity. In the normal hemispheres of both OTX groups (n=294), the ocular dominance distributions did not markedly differ from their respective control animals (n=395). The overall responsiveness was initially reduced in all the OTX cats, with the non-deprived ones showing a gradual increase with recovery time. The percentage of cells selective for orientation or direction was dramatically decreased, respectively in both the nondeprived ($\bar{X}_1$ = 45.5; 27.9) and deprived ($\bar{X}_2$ = 65.3; 43.1), as compared to their respective control cats ($\bar{X}_1$ = 81.9; 63.8/ $\bar{X}_2$ = 65.3; 43.1). All of the receptive fields (RF) mapped were within 10° at the projected vertical meridian with the majority in the ipsilateral visual field to the section; the small number of RFs in the contralateral visual field adds further support for a nasotemporal overlap in the retina.

Although interhemispheric transfer in OTX cats has been previously described (Choudhury et al., Q.J. Exp. Physiol., 50:241, 1965), these results show that there is a near-total inactivation of the callosal pathway for the transfer of primary visual information. This inactivation appears not to be related with a reduction in cortical binocularity, as previously reported for callosum transected cats (Payne et al., Science, 207: 1097, 1981); nor involved in restoring binocularity after the critical period for previously monocularly deprived cats. However, the degree and type of responsiveness of visual cortical cells still receiving direct visual input is affected by this post-critical callosal inactivation. These findings do not rule out the function of the corpus callosum in other higher order interhemispheric functions.

82.13 UNMASKING SILENT SYNAPSES IN CATS REARED WITH UNEQUAL ALTERNATING MONOCULAR DEPRIVATION. D.C. Smith, T.M. Reeves & R.N. Holdefer, Developmental Biopsychology Lab, Southern Illinois University, Carbondale, IL. 62901

Tieman et al., (Neurosci. Abst.,5,810,1979) reported that following rearing with unequal alternating monocular deprivation (AMD,8hrs. vs 1-hr) 79% of the cells in striate cortex are driven by the 8-hr eye, whereas only 16% are driven by the 1-hr eye. Nevertheless, most cells driven by either eye displayed normal direction and orientation selectivity. In the present study, we examined the effects of acute removal of the 8-hr eye of AMD-8/1 cats in an attempt to determine if this eye was in some way suppressing the influence of the 1-hr eye in striate cortex.

Single-unit recordings were made from striate cortex of four paralyzed and anesthetized AMD-8/1 cats. Electrode penetrations were angled 20° posterior to anterior down the medial wall of the lateral gyrus and an unbiased sampling technique was used. Prior to removal of the 8-hr eye, 87/103 cells (84%) responded to the 8-hr eye while only 18/103 (17%) responded to the 1-hr eye (two binocular cells were found making this sum over 100%). In three of these AMD-8/1 cats, the 8-hr eye was then removed. In each cat, the 1-hr eye immediately began driving practically every cell sampled (i.e., 61/70 cells - 87%). Further, for the responsive cells, essentially every one displayed direction-selective (98%) and orientation-selective (92%) receptive fields. We conclude that suppression is definitely an operative mechanism in cats reared with unequal alternating monocular deprivation; however, this suppression is not associated with a degradation in the receptive-field properties of the cells which retain a functional, yet suppressed, input from the less-experienced eye. This finding stands in marked contrast to the effects of nonalternating monocular deprivation. Supported by NSF-BNS-8002251 to D.C.S.

83.1 DISTRIBUTION OF A CHICK EMBRYO FACTOR PROMOTING GROWTH OF RETINAL AXONS. N.G. Carri and T. Ebendal*. Department of Zoology, Uppsala University, Box 561, S-751 22 Uppsala, Sweden.

Nerve growth in the CNS may be controlled by factors similar to those operating in cultures of peripheral nervous tissue. However, little is known about the activity and distribution of such factors in CNS.

We designed a short-term bioassay with the embryonic chick neuroretina to study factors controlling neurite extension. A dose-dependent outgrowth of retinal fibres in response to extracts of the optic lobe was established. Within a certain interval (encompassing three successive twofold dilutions) the length of outgrowing neurites showed a linear relationship (r=0.99) with the concentration of the extract. We defined a unit of growth activity based on the half-maximum outgrowth length. This allowed direct expression of the activity in units as a linear function of fibre length within certain limits. Under the condition that other tissue extracts follow a dose-response curve with a similar shape, their activity should be possible to determine by a series of 8-fold dilutions. We used this as a basis to study the level and distribution of neurite extension-promoting activity in other parts of the chick brain. Peripheral tissues of the chick embryo were also examined.

The results show that activity is present in other parts of the brain and even to a greater extent in peripheral tissues.

Several explanations can be offered to account for this apparent low level of target specificity in the stimulation of optic axons. Possibly, factors with a unique presence and function in the optic lobe can in the bioassay be replaced by other related factors with a different distribution. Alternatively, a factor common to a variety of tissues, including the optic tectum, may exist and possibly function to regulate fibre growth in general in the CNS and PNS.

(N.G. Carri is a Fellow of CONICET, Argentina.

83.2 NEURON PERFORMANCE IN VITRO IS ENHANCED BY AUTOLOGOUS CONDITIONING OF THE CULTURE ENVIRONMENT. R.J. Riopelle and D.A. Cameron*. Dept. of Medicine (Neurology), Queen's Univ., Kingston, Canada K7L 2V8.

Both fasciculation in vertebrate neurogenesis, and axonal elongation in juxtaposition to pioneer neurons in insect neurogenesis suggest that the axonal surface and/or the peri-axonal environment are favourable to process formation. Specific target reconnectivity following axotomy might be facilitated if the original axon modulated its matrix to provide a favourable milieu for the regenerating axon. Using an in vitro paradigm, environmental conditioning by neurons for enhanced neuronal performance has been examined. Neuron-enriched cultures of dissociated 8 day chick embryo spinal cord and optic lobe were established on a polylysine substrate in defined medium. Following 7 days of culture, the medium was harvested and the cells removed by gentle agitation in a Ca^{++}-Mg^{++}-free buffer. Thereafter, freshly dissociated neuron-enriched spinal cord and optic lobe cells were seeded at low concentrations onto the plates and the appropriate 0.2 μ millipore-filtered defined medium added back to the plates. At various times in culture thereafter, process length of neurite-bearing cells was measured. Both spinal cord neurons and optic lobe neurons have longer processes in neuron-conditioned environments than in control environments, indicating autologous but non-specific environmental conditioning; the effect is not related to enhanced attachment of neurons to conditioned substrate. To examine for interregional specificity of conditioning, the performances of neurons in homotypic and heterotypic environments was compared. For both spinal cord and optic lobe neurons, the performances were generally better or as good in homotypic environments, indicating additional qualitative interregional differences in conditioning factors specific to the neuron populations examined. The enhanced neuronal performance in autologous conditions demonstrated in these experiments suggests that indirect axon-axon interactions mediated via the environment could facilitate directionality, fasciculation, and specificity of reconnection.

83.3 RESPONSES OF RETINAL NEURONS TO PNPF, A SUBSTRATUM-BOUND NEURITE-PROMOTING FACTOR, Ruben Adler. Dept. of Biology, Univ. California at San Diego, La Jolla, CA 92093.

PNPF is a macromolecular factor which binds to polyornithine substrata and from that location stimulates neurite production by ciliary and other PNS ganglionic neurons. Some chick spinal cord cells have so far been the only CNS neurons found responsive to PNPF (Adler et al., Brain Res. 206: 129, 1981; Longo et al., Dev. Brain Res. 3: 277, 1982). Retinal neurons, which belong to the CNS, are reported here to be sensitive to the neurite-promoting effects of PNPF.

Neurite development of dissociated 8-day chick embryo retinal neurons was qualitatively and quantitatively evaluated upon cultivation on two different substrata. "PNPF(+)" substrata were prepared by exposing polyornithine-coated tissue culture dishes to medium containing 10% fetal calf serum and 25% rat schwannoma conditioned medium (a source of PNPF). This conditioned medium was omitted from the incubation medium in the case of "PNPF(-)" substrata. The dishes were thoroughly rinsed before cell seeding. In most experiments retina cells were seeded in serum-free "N1"-supplemented medium (Bottenstein et al., Exptl. Cell Res. 125: 183, 1980).

Neurite stimulation by PNPF could already be detected after 6 hr culture when approximately 45% of the attached cells showed neurites on PNPF(+) as compared with 5-7% on PNPF(-) substrata. At 3 days in vitro PNPF(+) cultures still contained more neurite-bearing neurons, many of which had longer neurites than those in PNPF(-) cultures. Six-hour cultures were used as a convenient assay to further analyze retina neuronal responses to PNPF. Exposure of PNPF(+) substrata to concanavalin A before cell seeding inhibited neuritic responses to PNPF. This inhibitory effect was concentration-dependent and could be prevented if concanavalin A treatment was followed by α-methylmannoside before cell seeding. As observed in previous work with ciliary neurons (Adler et al., Dev. Brain Res., in press) pretreatment of PNPF(+) substrata with wheat germ agglutinin, which reacts with PNPF in affinity chromatography experiments, failed to inhibit neurite responses of retinal neurons to PNPF. In fact, PNPF(-) substrata actually gained some neurite promoting properties after exposure to wheat germ agglutinin. Fetal calf serum had a marked inhibitory effect on neurite responses to PNPF when added to the medium in which the cells were cultured. This inhibitory effect was not observed when serum was only used for substratum pretreatment and the cells were cultured in serum-free medium.

Besides supporting the notion that at least some CNS neurons are sensitive to PNPF, these data indicate that neurite production by retinal neurons can be regulated by different signals, both stimulatory and inhibitory. SUPPORTED BY USPHS GRANT EY-02854.

83.4 A HEPARAN SULFATE PROTEOGLYCAN (HeS-PG) ON THE SURFACE OF NEURONS IN VIVO IS ASSOCIATED WITH A POTENT INDUCER OF IN VITRO NEURITE OUTGROWTH. W.D. Matthew*, A.D. Lander*, R.J. Greenspan, & L.F. Reichardt, Dept. Physiology, University of California, San Francisco, CA 94143.

Previous work in our laboratory has shown that a HeS-PG secreted by corneal endothelial cells is associated with a potent inducer of neurite outgrowth (Lander et al.1982.J.Cell Biol.). PC12 cells synthesize membrane-bound and secreted forms of a similar HeS-PG that after adsorption to polycationic surfaces also induces rapid neurite outgrowth by many types of neurons. It is not yet clear whether the outgrowth-promoting activity requires only the HeS-PG or requires in addition other molecules non-covalently associated with it. Five hybridoma cell lines have been isolated that secrete monoclonal antibodies with different specificities that bind either the HeS or core protein portions of the HeS-PG. The antibodies have been used to purify the HeS-PG, which consists of an 80 kdalton core protein and approximately 3-15 kdalton HeS chains. They have also been used to isolate a variant PC12 cell line that does not secrete an active neurite inducer and does not synthesize a normal HeS-PG. Studies using heparinase and trypsin imply that both the HeS and protein are required for biological activity in vitro. The HeS is clearly required for efficient adsorption to polycationic surfaces and may have no other function. Protein is clearly required to anchor the HeS-PG to the cell surface and is almost certainly required to induce neurite outgrowth. By immunocytochemical criteria, the HeS-PG is on the surface of neurons, but not other major cell types in the PNS, and is on the surface of neurons and at least some astrocytes, but not other major cell types in the CNS. During development of the rat Superior Cervical Ganglion, the HeS-PG level increases dramatically between 1 and 2 weeks postnatally (Greif and Reichardt.1982. J.Neuroscience), roughly the same time as one observes axon invasion, dendrite elaboration, synaptogenesis, and increases in neurotransmitter enzyme levels.

HeS-PG's are prominent constituents of basement membranes, neuronal tissues, and target tissues. These results provide evidence that some types of HeS-PG, possibly in association with other molecules, have powerful effects on neuronal development in vitro, and are appropriately placed to have similar effects in vivo.

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83.5 MORPHOLOGICAL AND GROWTH CHARACTERISTICS OF CELLS FROM THE NEURON-LIKE CLONE PC-12: CHEMICALLY INDUCED CELL FUSION AND EFFECTS OF NERVE GROWTH FACTOR. Raj Kapur, Susanne Huttner, and Paul O'Lague
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Large numbers of PC-12 cells in culture readily fuse after treatment with polyethylene glycol (PEG) to form multinucleate cells (O'Lague, PH, and Huttner, SL, PNAS 77:1701-1705). These fused cells (some up to 250 μm in diameter) exhibit many properties of their unfused counterparts including the ability to extend neurite-like processes in response to nerve growth factor (NGF). To determine the effect of multiple nuclei on the growth properties of these cells we have compared at the light microscopic level the responses to NGF of both the fused cells and unfused parent cells grown in culture. The results of this study indicate among other things that fused PC-12 cells in the presence of NGF (7s, 100 ng/ml) grow more processes per cell and extend processes at a greater rate than unfused (mononucleate) PC-12 cells. Moreover, the magnitude of these responses is proportional to the number of nuclei (e.g., mean number of cell processes/cell= 2.1 ± 1.0, n=82 cells, for mononucleate cells versus 8.5 ± 4.4 for cells with 9-12 nuclei, n=40; mean process length for 3 days growth in NGF = 66 ± 40.2 μm for mononucleate cells, n=166 processes, versus 126 ± 98.0 for cells with 9-12 nuclei, n=383 processes; ±=standard deviation). These differences in responses between fused and parent cells are not a result of PEG treatment because cells plated at low density and exposed to PEG do not fuse and yet they exhibit growth characteristics that are similar to those of the unexposed parent cells.

Possible explanations for the observed differences in responses to NGF include, among others, increased cytoplasmic volume, increased surface membrane area, and increased gene dosage. Experiments designed to alter the nuclear/cytoplasmic ratio by enucleation may prove useful in determining the role of multiple nuclei in these growth differences.

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83.6 POSTNATAL DEVELOPMENT OF CORPUS CALLOSUM AND ANTERIOR COMMISSURE IN THE MOUSE. G.O.Ivy and G.S. Lynch, Department of Psychobiology, University of California, Irvine, CA 92717

Several studies on rat and cat have shown that the neurons which send axons through the corpus callosum (CC) are widely distributed during the early neonatal period, but later become confined to more discrete cortical areas. We decided to investigate this phenomenon in the mouse and, in addition, to determine if the cells which project through the anterior commissure (AC) undergo similar distributional changes.

First, the distribution of cells of origin of the CC was examined in day 0 to 7 (n=12) and day 20 or older (n=7) C57Bl/6 mice. Multiple injections of horseradish peroxidase (HRP: 50-60% w/v in dist. water with 2% DMSO and 5% nonidet) were placed unilaterally throughout the neocortex of anesthetized mice. After a survival time of 12-30 hrs, the animals were perfused and the brains processed with benzidine dihydrochloride. We have found that the neurons which project through the CC in the mouse undergo distributional changes which are similar in both pattern and in time course to those reported in the rat (Ivy and Killackey, '81). From day 1 to day 3, labeled cells are found in two continuous bands in laminae Va and Vc-VIa. From day 4 to day 6, cells in layers IV, III and II become progressively labeled; layer IV of the barrelfield area becomes distinguishable by its relative lack of label. By day 20, neurons in certain areas no longer transport HRP from the contralateral cortex and so the adult pattern of more discrete callosal zones is established.

In the second part of the study, the distribution of cells of origin of the inferotemporal component of the AC was investigated in day 0 to 6 (n=17) and adult mice (n=7). The procedures were as described above, however the CC and hippocampal commissure were transected just prior to the HRP injections into the ventral cortex of one hemisphere. At day 1, HRP labeled cells extend rostrocaudally through the insular cortex and adjacent neocortex, from a level just posterior to the AC crossing back through occipital cortex. These cells are located mainly in laminae IV and lower V in neo- and insular cortex, and these labeled laminae converge just ventral to the rhinal fissure. By day 4, another population of cells becomes labeled for the first time. These are located in laminae II and III of the pyriform cortex; there are also a few layer III cells labeled in neo- and insular cortices. The areal and laminar patterns of cells seen at day 4 do not differ signficantly from those seen in the adult, although there may be fewer cells labeled in the adult. Finally, both the cortical nucleus and the posterior portion of the lateral nucleus of the amygdala are labeled as early as day 1.

In summary, in contrast to the CC, the AC does not appear to develop from an initially wider expanse of cortex.

83.7 THE GROWTH OF CERVICAL AXONS TO THE MAMMALIAN DIAPHRAGM DURING DEVELOPMENT. D.F. Davey, P.G. Noakes* and M.R. Bennett*, Department of Physiology and Neurobiology Research Centre, University of Sydney, N.S.W. 2006, Australia.

A study has been made of the growth of motor nerves to the rat diaphragm, from the 3rd, 4th and 5th cervical segments (C3, C4 and C5) which contain the phrenic nucleus. Foetal Wistar rats were fixed for light microscopy and serial sections were cut transversely to the body axis. Some embryos were prepared for combined light and electron microscopy. Thick and thin serial sections were cut; the thick sections were stained for light microscopy and the thin sections were prepared for electron microscopy. The serial light microscopic sections were used to reconstruct the structure of the sectioned region of the embryos with the aid of a computer. Electron microscopy was used to confirm the identity of small nerve bundles near the limit of resolution of the light microscope.

The cervical and thoracic regions of embryos from 11 to 17 days embryonic age have been examined and reconstructed. At 11 days embryonic age, the spinal roots C3, C4 and C5 have projected about 100um onto the left and right cardinal veins. On each side, these roots associate into a single nerve trunk and by 11.5 days, these trunks have grown about 250um down the cardinal veins. At this time the veins are located laterally on either side of the spinal cord and extend the full length of the trunk. These nerves, now identifiable as the phrenic nerves, continue to grow along the cardinal veins through embryonic days 12 and 13. By fourteen days embryonic age, the trunks have grown over 1500um along the cardinal veins and then diverge to grow into the pools of pre-muscle cells of the diaphragm.

This pattern of growth is not in accord with most descriptions of phrenic nerve development which suggest that the phrenic nerves enter the presumptive diaphragm before it migrates to its position in the lower thorax. The growth appears initially to be determined not by the position of the target, but rather by growth in association with the cardinal veins. The adhesion of cholinergic neurons to embryonic blood vessel tissue is greater than that to other tissues (Freeman, Stratford, O'Rourke and Bennett, Proc. Aust. Physiol. Pharmacol. Soc. 12: 156P, 1981). This suggests that the phrenic nerves follow the cardinal veins as a result of preferred adhesion.

83.8 COLLATERAL SPROUTING AND REGENERATION OF RABBIT CORNEAL NERVES FOLLOWING EXPERIMENTAL WOUNDS. A. J. Rózsa, R. W. Beuerman, and R. B. Guss*. Louisiana State University Eye Center, New Orleans, LA 70112

Circular (4 mm dia) and radial linear wounds were produced bilaterally on the corneas of 39 albino rabbits (2-3 kg). The animals were killed at designated postoperative intervals (8 hrs to 30 days). The remodeling of neural organization in the epithelium was observed on gold chloride-stained corneas that were mounted flat for light microscopy.

Sixteen hours after wounding, degeneration of intraepithelial terminals just outside the margin was paired with emerging sprouts from the superficial plexus. Degeneration occurred in a disto-proximal fashion ending about 72 hours after wounding. The normal intraepithelial terminals were replaced by an organized pattern of collateral sprouts which coursed through the unwounded epithelium perpendicularly to the wound margin. The origin of these densely-packed neurites could be located clearly in the intact subepithelial neural plexus at up to 3 mm from the edge of the wound. These wound-oriented neurites were much longer and thicker than normal terminals and reached a maximum growth at 3 to 4 days after wounding. Neurites began degenerating at 7 days post-wounding and the growth of a second "wave" of neurites was observed.

The origin of the second generation neurites was the transected stumps of lesioned pre-terminal axons of the superficial nerve plexus, located beneath the epithelium. These regenerating axons were mixed with the degeneration debris of the first "wave" of wound-oriented neurites and were morphologically distinct from them in that they appeared thinner, shorter (250 μm maximum), and more densely packed around the wound margin. The disposition was seen to be oblique to the wound and approached the organization of normal terminals. The organization of intraepithelial innervation around the wound appeared to return to normal by 30 days after wounding.

The regenerative terminal endings appeared to follow the epithelial cells and formed into dense and disorganized neuroma-like arrangements. Removal of a disk of epithelial tissue from within the limits of the circular wounds allowed more extensive lateral movement for the reepithelializing cells. In such wounds, the regenerating terminals appeared to be influenced by the migration of epithelial cells and followed relatively straight and centripetal paths deep into the reepithelialized wound. In either case, the pattern of nerves within the wound persisted beyond the time at which the tissue surrounding the wound returned to normalcy. The migration of epithelial cells may represent a guidance system for the extension of neurites.

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83.9 MORPHOLOGY OF GROWING AXONS IN THE MOUSE CEREBELLUM. C.A. Mason. Dept. of Pharmacology, New York Univ. Sch. Med., New York, NY 10016.

The morphology of developing cerebellar climbing (CF) and mossy (MF) fibers was studied during the transition from neurite with single growth cone to mature axon arbor. Axons in mice of 1-30 postnatal days were labelled with horseradish peroxidase (HRP) by two methods: (1) Animals were anaesthetised, and HRP solution was injected using a glass micropipette with a 10-30 µm tip into the white matter of the cerebellum. (2) Fresh 600-800 µm slices of cerebellum attached to brain stem were placed in an oxygenated buffered salt solution. A crystal of HRP carried on a 10-20 µm micropipette tip was inserted into the inferior or middle cerebellar peduncle or into the white matter of a single folium. Thirty minutes after injection, routine aldehyde fixation was performed by perfusion of animals or immersion of slices. Vibratome sections were processed using the DAB procedure for light and electron microscopy, as previously described (Mason, 1982, Neurosci. 7:561).

During early postnatal development, MF bear large broad growth cones (10 µm), with many filopodia, while CF have smaller, more slender and foliate growing tips. MF growth cones occur linearly on single unbranched fibers, whereas branching of CF often occurs at growth cones. As late as 14 days, terminal structures on both CF and MF that still have the shape of growth cones as seen in the light microscope, make synaptic contacts and contain accumulations of synaptic vesicles. Both MF and CF can simultaneously bear mature and immature terminal forms, and also have spikey projections and free-ending filopodia proximal to maturing terminals. The maturation of shape and synaptology of MF and CF terminals takes at least three weeks.

CF that have not yet reached the Purkinje cell layer can be highly branched and covered with growing tips. By the end of the first postnatal week, branches of single CF extend over multiple Purkinje cells and give off feathery growing tips towards two or more cells, confirming electrophysiological findings on multiple innervation of Purkinje cells by immature CF (Crepel et al., 1976, J. Neurobiol. 7:567). These experiments hold promise for suggesting rules for several aspects of neural development: the role of the postsynaptic target in determining characteristic axonal forms, the synaptic relations of supernumerary branches and the events during their subsequent regression. (Supported by NIH Grant NS-16951).

83.10 NEURITES OUTGROWING FROM THE RETINAL EXPLANT PREFER CO-CULTURED TECTAL TISSUE TO CEREBELLAR TISSUE IN VITRO. Myong G. Yoon and Frank A. Baker*. Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada, B3H 4J1.

Possible trophic interactions between the neurites outgrowing from the retinal tissue and different types of brain tissues, explanted from adult goldfish, were tested in vitro by co-culturing various combinations of the neural explants. When an elongated rectangular piece of the retina was dissected free along the naso-temporal axis near the equator, and implanted on a culture dish in predesignated orientation, the neurites sprouting out from the retinal explant initially grew in the direction towards the phantom optic disc. In the absence of a target tissue, however, these retinal neurites tended to curl up to form clockwise spirals as they grew further on the poly-L-lysine coated culture dish. If a piece of tectal tissue, dissected from the topographically matching area, was introduced to the retinal culture, the curling retinal neurites changed the pattern of their outgrowth; a majority of the surviving retinal neurites became straight to grow preferentially towards the tectal tissue. This observation suggests that the co-cultured tectal tissue may emit diffusible trophic factors, which attract the retinal neurites to grow towards the tectal tissue.

To test whether the trophic attraction of the retinal neurites is specific to their proper target tissue (i.e. the optic tectum) or general for any brain tissue (e.g. the cerebellum), the retinal tissue is co-cultured with the tectal tissue and the cerebellar tissue at the same time. In most cases, the retinal neurites grow preferentially towards the tectal tissue rather than to the cerebellar tissue. The result suggests that the trophic attraction of the retinal neurites is specific to the tectal tissue. (Supported by grants from MRC and NSERC of Canada.)

83.11 GUIDEPOST NEURONS: PATHFINDING BY GRASSHOPPER PERIPHERAL PIONEER NEURONS AND, FOLLOWING REMOVAL OF PIONEERS, BY NON-PIONEER NEURONS. Haig Keshishian, David Bentley, Alma Toroian-Raymond*, and Dawn Yokoe* Neurobiology Group and Department of Zoology, University of California, Berkeley, CA 94720.

The first neurons to appear in the grasshopper leg during embryogenesis are a pair which lie near the limb tip and project axons to the CNS along a stereotyped pathway(Bate,Nature,260: 54, 1976). These cells do not retain a dendrite in the epithelium, although later pioneers in the tarsus do so, and within the CNS they project at least one fiber anteriorly in the ipsilateral connective. Bate suggested that the growth cones of these "pioneer" neurons might navigate along a chain of cells whose placement would comprise the pathway. We report the following observations in support of this hypothesis: (1) at three sites in the path a cell or a cell-pair (here termed F1, F2, and CT1) is located which is distinctive first in eventually differentiating into a neuron, and secondly (for F2 and CT1), in binding a neuron-staining antibody (Jan & Jan,PNAS 79: 2700, 1982) before the arrival of the pioneer growth cones;(2) antibody staining and dye injection reveal that the pioneer growth cones navigate from cell to cell along this route, and that the two abrupt turns in their axon trajectory occur at the sites of F2 and CT1. TEM shows direct membrane apposition between the pioneers and at least cells F1 and CT1. SEM shows that the growth cones navigate along the inside of the epithelium, and that no continuous morphological feature (groove or ridge) marks the path. (3) the pioneers form dye-passing junctions with each of these cells, and with no other cells along their route. Later, nerve branch points occur where axons join the path, e.g. nerve 3, femoral chordotonal and subgenual axons project onto F2 , F1, and the pioneer somata, respectively. Pioneer growth cones could locate successive cells in the chain by undirected filopodial exploration. The growth cones have profuse filopodia which sweep a large area within the limb (at one point spanning the lumen) and which are long enough (at least 75 microns) to cross the intervals between cells. That extrinsic information orienting the initial extension of filopodia from the cell body is not present, is indicated by morphogenesis of neuron F1. Before axonogenesis, this neuron extends many radially disposed filopodia which are not oriented in the direction which the axonal growth cone will eventually take.

To determine whether the pioneers have a path following capability not shared by other neurons, we injected the first pioneers with Lucifer Yellow dye, before the onset of axonogenesis, and killed them by photoinactivation. Embryos were cultured with yolk cells to 40-45%, and the paths of neurons F1 and F2, which normally follow the pioneers, were examined by antibody staining and dye injection. In the absence of the pioneers, F1 extended filopodia, initiated axonogenesis, contacted F2 (the nearby cell which would have been contacted by the pioneers), became dye-coupled to it, and established a normal axon trajectory, as did F2. Thus, other neurons are able to follow the pioneer pathway.

83.12 THE ANTI-RETINOTOPIC ORGANIZATION OF THE OPTIC NERVE IN THE FROG. F. Scalia and V. Arango* (Spon: W. Riss) Dept. of Anatomy and Cell Biology, Downstate Medical Center, Brooklyn, N.Y. 11203.

The argument (e.g., Horder and Martin, 1977) that chemoaffinity is not necessary for the formation of a normal retinotectal projection has been promoted by data suggesting that mechanical guidance is sufficient. That is, the optic nerve and optic tract appear to be, themselves, retinotopically organized in certain species, or organized in a topologically conservative pattern that can be conceptually transformed into normal retinotopic order by operations on the group rather than on the individual axons. However, while such data do not disconfirm the chemoaffinity hypothesis, finding the optic nerve to be anti-retinotopically organized in a single species would present a significant counter-example to mechanical guidance. An anti-retinotopic pattern is non-topological in at least one of the two dimensions of the retina, and its normalization requires the sorting of individual axons, e.g., were the retina mapped folded along its dorsoventral meridian, with nasal and temporal axons intermingled.

Incisions were made in the retina in 14 adult Rana pipiens in varied locations around the optic disc, and HRP was introduced into the lesions. Severed, HRP-filled axons were traced through the optic nerve and optic tract to the optic tectum by a combination of whole-mount and serial-section analyses. Axons arising from the dorsal sector of the retina were located on the dorsal side of the optic nerve throughout its length. They coursed in parallel within the ventral margin of the optic tract and terminated on the lateral side of the tectum. Ventral-sector axons occupied the ventral side of the nerve and the dorsal margin of the tract, and ended in the medial tectum. Temporal axons occupied both the nasal and temporal sides of the optic nerve, but formed a single fillet in the middle of the optic tract, ending in the rostral tectum. Nasal axons also occupied both sides of the nerve, and continued along both margins of the optic tract to reach the caudal tectum. Axons filled by nasal or temporal lesions made close to the optic disc tended to bridge the center of the optic nerve cross-sections and to extend toward the deep side of the optic tract. Otherwise, peripheral retinal axons occupied peripheral locations in the nerve and tract.

While we cannot measure the degree of ordering in this system from the available data, it is clear on a gross scale that the optic nerve carries a folded, reduplicated, anti-retinotopic map of the retina, in which nasal and temporal axons are mixed, whereas the optic tract carries a topologically normal but split representation (Gaze and Grant, 1978). The former is transformed into the latter at or near the optic chiasma by a mechanism that sorts axons of nasal and temporal origin. (Supported by NSF grant BNS 79-23788.)

83.13 SPECIFICITY AND CHARACTER OF NEURITE OUTGROWTH ON DEFINED CELL TYPES. J.R. Fallon* and M.C. Raff* (SPON: G. McKhann). MRC Neuroimmunology Project, Dept. of Zoology, University College London, London, WC1E 6BT, U.K.

While neurite outgrowth on artificial substrates has been studied extensively, comparatively little is known about the character and specificity of neurite extension on identified cells in vitro. We have compared neurite outgrowth from CNS and PNS explants on monolayers of highly enriched astrocytes (>95% as identified by antibody staining for the glial fibrillary acidic protein, GFAP), to that on skin fibroblasts.

Explants from rat superior cervical ganglion (SCG, 18-21 days gestation), spinal cord (SC) and retina (both 13-14 days gestation) were cultured under conditions where few explant-derived cells contributed to the monolayer population. Neurite outgrowth was monitored by phase microscopy and by staining with an anti-neurofilament antibody. Neurite outgrowth from the three different types of explants was much greater on astrocytes than on fibroblasts. For example, in the first 24-48 hours after plating SCG neurites extended 1.5-3X further on astrocytes than on fibroblasts, and while SC and retinal neurites showed no appreciable growth on fibroblasts, they grew vigorously on astrocytes. Furthermore, neurites from all three types of explants studied grew singly or in small fascicles on astrocytes, while SCG neurites formed large bundles on fibroblasts.

Several types of experiments suggested that the astrocyte cell surface, rather than soluble molecules secreted by astrocytes were responsible for promoting neurite extension: 1) In mixed monolayer cultures SCG neurites grew preferentially on the astrocytes; 2) SCG neurite outgrowth on fibroblasts was uneffected by the presence of astrocyte conditioned medium and 3) polylysine coated dishes pre-treated with conditioned medium from astrocytes or fibroblasts did not promote retinal neurite outgrowth. It seems likely that the preferential growth of neurites on the surface of astrocytes in culture reflects the tendency of neurons and their processes to migrate along the surface of astrocyte-like cells during neural development. If so, then one can begin to study the molecular mechanisms of this neuronal glial interaction.

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83.14 HIGH VOLTAGE ELECTRON MICROSCOPY (HVEM) OF GROWTH CONES SHOWS THE PRESENCE OF VESICLES INTERCONNECTED BY A NETWORK OF FILAMENTS. H.T.Tsui, H.Ris* and W.L.Klein. Interdepartmental Program in Neuroscience, Northwestern University, Evanston, IL 60201 and Department of Zoology, University of Wisconsin, Madison, WI 53706.

High voltage electron microscopy of unsectioned cultured neurons prepared with the critical point drying procedure provides an excellent overview of integrated cytoplasmic structures. Dissociated cells from 8-day old embryonic chick retina were grown on polylysine- and formvar-coated gold grids. After 3-6 days in culture, the cells were fixed, dehydrated and critical point dried with CO_2. The ultrastructure of the growth cones was examined with the 1 MeV microscope at the Madison HVEM facility. Stereo micrographs showed that in all areas of the growth cones there was a predominant network of filaments of uniform thickness (4-8 nm). Interconnections between endoplasmic reticulum, microtubules and the filamentous network were very common. Most strikingly, there were a large number of vesicles interconnected by the filamentous network throughout most of the growth cones. At the proximal part of the growth cones, the vesicles were of irregular sizes (25-75 nm) and they often formed clusters associated with the smooth endoplasmic reticulum. At the edge of the growth cones, the interconnected vesicles were of uniform sizes (35-40nm). These vesicles also extended into the filopodia. In younger cultures, there were fewer vesicles at the edge of the growth cones and more clusters of irregular-sized vesicles. Stereo micrographs also were taken from 0.25 µm thick sections of plastic embedded monolayer retinal cultures. Vesicles were observed at the edge of growth cones, but they appeared to be of larger sizes (45-50 nm). The filamentous connections among vesicles were also visible but less distinct than in critical point dried neurons. These interconnected vesicles may exist in developing neurons to supply membranes and other proteins at the tip of the neurites. Alternatively, these vesicles may be formative synaptic vesicles. Their size approximated that of synaptic vesicles, and they were more prevalent in older cultures. In either case, the structural connections of the vesicles with the filamentous network may serve important functions in neurite development and synapse formation.
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83.15 APPLICATIONS OF A MICROCOMPUTER-BASED VIDEO ANALYZER: NEURITE OUTGROWTH RATES AND DENSITOMETRY. T. Ford-Holevinski*, N. S. Radin* and B. W. Agranoff. Mental Health Res. Inst. and Dept. of Biol. Chem., Univ. of Mich., Ann Arbor, MI 48109.

The quantitative measurement of neuronal outgrowth in explant culture usually employs indices in which estimates of outgrowth density and neurite length or area occupied by neurites are incorporated into a formula for outgrowth. This is tedious and imprecise, requiring many readings for each data point. While simplification of such growth assays has been attempted, there is not at present a generally applicable alternative. We present preliminary studies on the development of an automated assay which employs an Apple II microcomputer, an inexpensive digitizer (The Dithertizer II, Computer Station Inc., St. Louis, MO), and a 128K memory card (Legend Industries, Detroit, MI) to capture and analyze images of growing goldfish retinal explants. The video image of a low-power magnification of the explant is digitized in a matrix of 280 x 192 single bit pixels. By repetitive scanning at various intensity thresholds, an eight bit image is constructed within the memory card. Image enhancement techniques can then be applied to this image (e.g., background subtraction to correct lighting and video camera irregularities). Suitable thresholds are then selected to maximize neurite visualization and eliminate the central explant in the one bit Apple image display. Further enhancement, such as restoration of neurite image continuity and removal of scratches and debri from the image, is accomplished manually on an Apple graphics tablet. Automated image processing and pattern recognition schemes now being explored may eliminate the need for this latter step. Neurite outgrowth is then expressed as total pixels occupied. All software is written in 6502 machine language.

We have found this system is also useful for quantitative densitometry. Developed chromatographic spots or bands visualized by autoradiography, staining, charring, etc., are typically darkest in the center and feathered toward the periphery. Since density may not be a linear function of the amount of substance at a given point, precise quantitation requires multiple sampling measurements. The use of absorbance standards, followed by computational linearization of each picture element's intensity is employed to solve this problem. (Supported by Grants NS 03192 and NS 13743.)

83.16 REAL-TIME MEASUREMENTS OF MINUTE NEURONAL CURRENTS WITH A CIRCULARLY VIBRATING MICROPROBE. John A. Freeman, Bruce Mayes*, G. Jack Snipes*, and John P. Wikswo* Depts. of Anatomy and Physics, Vanderbilt University, Nashville, TN 37232.

Many developmental and regenerative processes appear to be regulated by intrinsic ionic currents, the measurement of which has heretofore proved problematical. We have developed a miniaturized vibrating probe which should facilitate measuring minute currents from a variety of preparations, and which is particularly well-suited for studying ionic currents in cultured neurons and muscle. Our device consists of a shielded, platinized tungsten microelectrode which is moved in a circle of 15-20 µm diameter at 500 Hz by two miniature loudspeakers mounted at right angles, in turn driven by sine and cosine voltages generated by a PDP 11/34 computer. The microelectrode voltage $V(\theta)$, measured by an ultra-low-noise amplifier, is averaged over a number of cycles at each of 256 values of the angle θ around the circular path. The rapidity of motion and sampling essentially eliminates low-frequency electrode noise. By expressing the voltage of the point (x, y) in terms of a 2-dimensional Taylor's series expansion of the potential at the central point (x_o, y_o), the current density in the medium at the central point can be shown to have components J_x and J_y given by the convolution integrals

$$J_x = -\frac{1}{\rho r}\int_0^2 V(\theta)\cos\theta d\theta \qquad J_y = -\frac{1}{\rho r}\int_0^2 V(\theta)\sin\theta d\theta$$

where ρ is the resistivity of the medium, and r is the radius of the circle. The gradients of J at (x_o, y_o) are obtained similarly from the second-order expansion terms. The computer evaluates these integrals in real time, resulting in a polar plot of the principal current density vectors and of a vector which points in the direction of the local current source. With this method, it is possible to measure current densities as low as 5 nA/cm^2. Use of this device has resulted in the discovery that the growth cones of growing goldfish retinal ganglion cell neurites generate steady-state currents of 15-30 nA/cm^2, which flow inward at the tips of the filopodia, and back outward at their bases. The circularly vibrating microprobe appears to be well-suited to a host of other applications. Supported by NIH Grant EY01117-10 to JAF.

83.17 EFFECTS OF SUBSTRATE CHARGE ON NEURITE GROWTH, ORIENTATION, AND ADHESION. G. Jack Snipes*, Bruce N. Mayes*, and John A. Freeman. Dept. of Anatomy, Vanderbilt University, Nashville, TN. 37232.

Little is known about the mechanisms that control the direction of neurite growth, or that affect the adhesivity of neuritic growth cones to the substrate upon which they are growing. These mechanisms must play an important role in neuronal growth and development, synaptogenesis, and during regeneration. It has been shown that cultured neurites from goldfish retinal explants grow in a clockwise direction on a positively charged surface (Heacock et al., Science, 1979). The basis for this directional growth selectivity is unknown. We have explored the role of substrate charge on neurite adhesion and direction of growth. Cultures of goldfish retinal explants were prepared 10-12 days after optic nerve crush using a modification of the method of Landreth and Agranoff (Brain Res., 1979). In order to control the surface charge of the substrate rigorously, different substrates were covalently bound to glass coverslips derivatized with 3-aminopropyltriethoxysilane (Gottlieb and Glaser, BBRC, 1975). Substrates tested included glutaraldehyde with or without BSA, poly-glutamate, poly-ornithine, and various lectins. All of these substrates, which differ radically in charge, were found to support neurite growth and extension. A semi-quantitative assay of neurite-substrate adhesion is provided by the shearing force generated by the vortex stirring action of a circularly vibrating microprobe (Freeman et al., Science, in press) vibrating at a diameter of 50 μm at 2500 Hz. A semi-quantitative assay of direction of growth is provided by measurement of a curvature index similar to that described by Hinkle et al. (J. Physiol., 1981.) We found that the greater the positive surface charge, the stronger the neurite substrate adhesion and the more pronounced was the tendency for clockwise growth. On negatively charged substrates neurite adhesion was relatively weak and non-uniform, although growth orientation was still clockwise. There thus appear to be two separate mechanisms: a non-specific charge interaction which affects adhesion but not direction, and a second mechanism which affects direction and which is independent of surface charge. The mechanism governing growth directionality is evidently intrinsic to the neurite, and might result from an asymmetry in the distribution or configuration of molecules associated with the growth cone. Supported by NIH Grant # EY01117-10 to JAF.

84.1 HISTOCHEMICAL EVIDENCE FOR INCREASES OF RNA IN THE DENERVATED NEUROPIL OF THE DENTATE GYRUS DURING REINNERVATION. B. Fass, J. Diggs* and O. Steward. Neurosurgery Dept., Univ. of Virginia School of Medicine, Charlottesville, VA 22908

We recently reported that an increase in incorporation of protein precursors occurs in the denervated neuropil of the dentate gyrus (DG) during reinnervation (Fass & Steward, Anat. Rec., 199: 80A, 1981). This increase may reflect protein synthesis within granule cell dendrites, since polyribosomes are redistributed within dendritic spines during the period of reinnervation (Steward & Fass, Neurosci. Abst., 7:474, 1981). The present study was designed to determine whether increases in RNA occur within the denervated zone during the period of increased incorporation.

Adult male albino rats were given a unilateral lesion of the entorhinal cortex (which is the major source of afferents to the DG) and survived for 2,4,6,8,10,12,15, or 20 days. Fresh cryostat-cut sections were mounted on microscope slides, fixed with Carnoy's solution, and stained with either methyl green/pyronin G (MGP; stains for nucleic acids), pyronin G (P; stains for RNA and low polymers of DNA; Kurnick, J. Gen. Physiol., 33:243, 1950), or methyl green (MG; stains for DNA and was used to check the specificity of P for RNA). Adjacent sections were incubated in ribonuclease A (Sigma type IIA; 0.5% solution) or deoxyribonuclease I (Sigma DN-ST; 0.5% solution) for 3-6 hr prior to staining with MGP, P, or MG.

In the control DG, the intensity of MGP staining appeared homogeneously pale throughout the neuropil relative to glial cells or to the granule cell layer. RNAse treatment markedly reduced the intensity of P staining in hippocampal pyramidal cells of CA2, CA3, and CA4, but less so in CA1 and granule cells of the DG. MG staining was not noticeably affected. DNAse treatment markedly reduced MG- but not P-staining. In the denervated DG, a similar pattern of homogeneously pale MGP staining was observed at 2 days postlesion. By 8 days postlesion, during the period of increased incorporation, a band of increased P staining appeared within the denervated zone. This band was not associated with glial cell bodies. RNAse treatment eliminated the band.

The altered intensity of P staining occurred at the same postlesion interval as the increased incorporation of protein precursors and the redistribution of ribosomes within dendritic spines (i.e., at 6-12 days postlesion). Since P is thought to stain RNA, the present results suggest an increase in RNA content within the denervated/reinnervated zone. We propose that granule cells redistribute some of their protein synthetic apparatus into denervated dendritic regions, thereby permitting a local regulation of protein metabolism within specific dendritic compartments.

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84.2 SYNAPTIC SPROUTING INTO MEMBRANE FILTERS IN ADULT NEOCORTEX. D. Kristt and R. McGowan*. Dept. Pathology, Div. Neuropathology, Stanford Medical Center, Stanford, CA 94305.

The formation of new synapses has been considered a potentially important factor in the process of recovery of function in the CNS. In past work the conclusive demonstration that a specific group of synapses was newly formed has been hampered by the intrinsic complexity of the adult CNS.

In the experiments described below membrane filter implants were made in either 30-35, or 120 day old rats. The animals were sacrificed 4-5 weeks later. Sterile 1 mm squares of Millipore filters (pore size: 5 μm) were placed into parasagittal knife wounds of frontal cortex. In toluidine blue stained plastic thick sections (1.5 μm), astrocytes and macrophages were occasionally seen within the filter. The adjacent brain along the depth of the implant was associated with only slight focal gliosis and dilatation of blood vessels. The ultrastructural examination revealed that a wide array of neuronal and glial processes had penetrated the implant. Implantation itself does not force neurites into pores. Many profiles were lollipop-shaped, with a narrow stalk attached to a bulbous varicosity (dia. 3-5 μm). The cytoplasm of these presumably dendritic processes was often relatively electron lucent and contained several large vacuoles and a plexiform array of microtubules (dia. ca. 25 nm). Small numbers of mitochondria were interspersed in the cytoplasm. In places on the plasma membrane, where these dendritic sprouts contacted other profiles, asymmetric junctional specializations were frequently found. The other neuronal processes, forming synapses with the dendrites, tended to have a slightly more electron-dense cytoplasm. In these presumably axonal processes microtubules were present and large numbers of 40-50 nm vesicles were often aggregated near the junctional membrane specialization. These presynaptic profiles range in size from 0.2-2 μm, dia. A few larger processes within the implant of irregular contour are seen which contain small numbers of the above noted organelles in addition to scattered ribosomes and rare multivesicular bodies. They are also likely to be dendritic in origin.

We conclude that neuronal sprouting into these membrane filters - both axonal and dendritic - does occur following the knife wound. These sprouts form synapses there, some of which appear to have features typical of asymmetric axodendritic synapses, seen in intact adult cortical neuropil. The principal advantage of this approach, is that it allows the observer to unequivocally identify new synapses, in vivo. Since the volume of the implant is known, quantitative analysis of synaptic sprouting is also possible.

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84.3 AXOTOMY CLOSE TO THE SOMA EVOKES DENDRITIC SPROUTING WITHOUT AXONAL REGENERATION IN LAMPREY CENTRAL NEURONS. G. Hall* and M.J. Cohen, Dept. of Biology, Yale University, New Haven, CT 06511.

The giant reticulospinal neurons (Muller and Mauthner cells) of larval lamprey have been observed in previous studies to respond to "distant" axotomy within the spinal cord (1-2cm from their somata) with an initial dendritic retraction by 4 weeks after transection. This was followed by limited dendritic sprouting starting at 8 weeks after axotomy (Fishman, 1975, Ph.D. Thesis, Yale Univ.). The proximal axon stumps of Muller neurons subjected to this form of distant axotomy have been reported to regenerate up to 1mm or more caudally by 4 weeks (C. Rovainen, 1976, J. Comp. Neurol., 168:545). In this study, we used intracellular injection of Lucifer Yellow to examine the 3 most rostral bulbar Muller cells in the larval sea lamprey Petromyzon marinus and determined the effect of cutting their axons in the hindbrain close to the soma (within 500μm). In contrast to the results of the previous "distant" axotomy studies, we found that "close" axotomy induced relatively rapid and extensive dendritic sprouting without noticeable axonal regeneration.

Neuritic sprouting from the dendritic trees of these bulbar cells was first observed at 15 days following close axotomy. Between 15 and 26 days, 10 of 13 lesioned cells showed a mean of 1.69 sprouts per cell. Some of these extended up to 1.3mm beyond the normal limits of the dendritic field. Of the 8 cells examined between 39 and 49 days, all showed extensive sprouting (mean number per cell, 7.56). No obvious dendritic retraction was observed. Regenerative sprouting from the axon stump was not seen, even at 49 days post axotomy. Sprouts were easily distinguishable from normal dendrites in that they were largely unbranched and frequently occurred in areas where normal dendrites from these cells are never seen. Most sprouts had swollen tips ranging in size from slight expansions resembling growth cones to extensive bag-like structures up to 60μm in diameter. Normal bulbar cell dendrites generally branch several times within 200μm of the soma and do not have swollen tips. Directions taken by the growing neurites were nonrandom, with 82% of the sprouts growing rostrally, caudally or caudolaterally. Three Mauthner cells examined 40 days after axotomy also showed dendritic sprouting.

Resting membrane potential and the size and shape of soma spikes evoked by intracellular current injections were not affected by close axotomy. Intact anterior bulbar cells contralateral to those axotomized were morphologically and physiologically indistinguishable from normal at all postoperative times.

We propose that the distance of the axonal lesion from the soma is a factor in determining whether the primary regenerative growth occurs in the proximal axonal stump or in the dendritic tree of the injured neuron.

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84.4 SPROUTING OF STRIATAL SEROTONIN NEURONS FOLLOWING NEONATAL 6-HYDROXYDOPAMINE. M.K. Stachowiak,* J.P. Bruno, A.M. Snyder,* R.G. MacKenzie, E.M. Stricker and M.J. Zigmond. Dept. of Psychol., Univ. of Pittsburgh, Pittsburgh, PA 15260.

Three-day-old rats given 6-hydroxydopamine (6-HDA)-induced brain lesions that deplete striatal dopamine (DA) by at least 90-95% show no obvious behavioral deficits, in contrast to the severe sensorimotor and ingestive dysfunctions that are observed when adult rats are given comparable lesions. Accordingly, we have begun to compare the biochemical consequences of 6-HDA administration in the developing and mature brain. We find that the long-term effects of 6-HDA injections (50-150 μg, ivt., 30 min after treatment with 25 mg/kg DMI, ip) in neonatal rats on striatal DA neurons is similar in several respects to those observed when 6-HDA is injected into adults. The DA depletions are permanent, and increases seen in turnover within residual DA neurons and the number of DA binding sites are similar regardless of when the lesions are made. On the other hand, there is a striking difference between the infant and adult rats in the effect of 6-HDA treatment on striatal serotonin (5-HT) neurons. When striata from neonatally lesioned rats were assayed 5-8 mo later there was a 123% increase in 5-HT levels and a 49% increase in the metabolite 5-hydroxyindoleacetic acid (5-HIAA) in rats with 95% DA depletions (Table 1). There were no such changes in the levels of 5-HT or its metabolite when comparable lesions were made in adults. Comparable increases in 5-HT and 5-HIAA following neonatal 6-HDA are present by at least 1 mo post-lesion.

		5-HT (μg/g)	5-HIAA (μg/g)
A. Lesioned as Neonates; killed 5-8 mo later	Control	.62 ± .04	.29 ± .02
	≥95%↓DA	1.38 ± .10	.44 ± .03
B. Lesioned as adults; killed 5-8 mo later	Control	.48 ± .03	.33 ± .04
	≥95%↓DA	.50 ± .05	.35 ± .02

There is also an accompanying increase in the velocity of high affinity [3H] 5-HT uptake. This increase is highly correlated with the increase in 5-HT levels, suggesting that sprouting of striatal 5-HT terminals has occurred in response to the degeneration of DA terminals. These changes in serotonergic neurons may be restricted to the striatum since there is no change in hippocampal 5-HT levels following neonatal 6-HDA.

We are now investigating whether these changes in striatal 5-HT terminals have any role in the sparing of behavioral deficits following large DA-depleting lesions in neonates.

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84.5 SYMPATHETIC FIBERS SPROUT CONCOMITANTLY IN THE CHOROID PLEXUS, PINEAL, THYROID AND SUBMAXILLARY GLANDS IN RESPONSE TO LESIONS IN THE STRIA MEDULLARIS. Zehava Gottesfeld and JoAnn McConnell. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77030.

We have demonstrated recently that lesions in the stria medullaris (SM) induce sprouting of sympathetic axon terminals in the medial habenula (MHb)(Gottesfeld et al., submitted). Lesions in the medial septal nuclei also result in proliferation of sympathetic axons in the MHb (1) as well as in the hippocampus (2) and in the pineal (3). The present work was undertaken to examine whether SM lesions induce noradrenergic (NA) sprouting in other fields of sympathetic innervation.

Sprague Dawley male rats (200 g) were used. Six experimental rats received bilateral high frequency lesions in the SM under chloropent anesthesia; six sham-operated rats were treated identically, but were not given lesions. After six weeks, the animals were sacrificed by decapitation and the brain, pineal, thyroid and submaxillary glands removed and processed according to the cryostat-glyoxylic acid method for fluorescence histochemistry to demonstrate catecholaminergic axon terminals and varicosities (4). All lesions were verified with thionine-stained sections.

Choroid plexus (CP): In sham-operated animals, some faint to moderately fluorescent (FL) fibers were scattered in the CP. In the rats with lesions, however, noradrenergic (NA) fibers were intensely and brilliantly FL and widely scattered in the CP.

Pineal (P): In sham-treated rats the P contained single FL fibers scattered throughout the tissue, while those with lesions, the FL fibers appeared in larger and more complex clusters.

Thyroid gland (T): In the sham-operated animals numerous FL fibers were located around blood vessels; fewer fibers with brightly FL varicosities were seen between the T lobules. A similar pattern was observed in the experimental rats, however, the number of fibers and the FL intensity appeared greater.

Submaxillary gland (SG): In general, NA innervation of SG in sham-treated rats was heterogeneous throughout the tissue. However, in the animals with lesions these fibers appeared more profuse and more intensely FL.

In view of these results, it appears that sympathetic nerve cells in the superior cervical ganglia respond to SM lesions by producing axon sprouts in their fields of innervation, remote from the lesion site. The mechanisms regulating this phenomenon require further investigation.

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84.6 RECOVERY OF ALTERNATION PERFORMANCE AFTER LESIONS OF THE ENTORHINAL CORTEX AND GRANULE CELL LAYER OF THE DENTATE GYRUS IN RATS. J.J. Ramirez*, L. Valentino*, J. Venditto*, and D.G.Stein. Dept. of Psychol., Clark Univ., Worcester, MA 01610, and Dept. of Neurol., Univ. of Mass. Med. Center, Worcester, MA 01605.

Entorhinal cortex lesions in rats denervate the granule cell layer of dentate gyrus; however, within 9-15 days after the denervation sprouting from several of the remaining intact afferents reinnervates the layer. The present study was designed to determine: 1) whether the granule cell layer is important for the performance of a spatial alternation task, and 2) the necessity of the granule cell layer after reinnervation in performing spatial alternation. Rats were given sham operations or bilateral entorhinal cortex lesions and tested for retention of a spatial alternation task. After the animals recovered to pre-operative levels of performance, they sustained bilateral injections of saline or colchicine (a neurotoxin selective for the granule cells of the dentate) into the dentate gyrus and were retested on the task. Rats with extensive damage to the granule cell layer, or to the granule cell layer plus entorhinal cortex, exhibited a transient deficit followed by recovery after approximately 15 days of training. The granule cells in the dentate gyrus contributed to the performance of spatial alternation as demonstrated by the initial impairment. However, neither the normal cells nor the reinnervated cells were critical for alternation behavior since the animals did eventually alternate at pre-operative levels.

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84.7 EFFECTS OF SCIATIC NERVE SECTION ON CAT SPINAL CORD SUBSTANCE P IMMUNOREACTIVITY AND DORSAL ROOT GANGLION CELL NUMBERS. A. Tessler, B. T. Himes*, M. Murray and M. E. Goldberger. Depts. of Neurology and Anatomy, The Medical College of Pennsylvania Philadelphia, PA 19129.

The reported loss of substance P (SP) in dorsal horn after peripheral nerve section may be due to loss of primary afferents, as after dorsal rhizotomy. We have examined the effect of transection of the cat sciatic nerve on: 1) SP immunoreactivity in the spinal cord; and 2) ganglion cell numbers in the L7 dorsal root ganglion (DRG). We used the unlabeled antibody (PAP) technique to demonstrate SP immunoreactivity and determined ganglion cell numbers by counting nucleoli in every fifth or tenth section of serially sectioned L7 DRG. Both L7 DRG were studied in normal, unoperated cats and in cats with unilateral sciatic nerve section. Raw cell counts were corrected for differences in ganglion cell volume and average nucleolar diameter. The right sciatic nerve of adult cats was ligated and cut in the lower third of the thigh, and a 1 cm piece of nerve just distal to the transection was resected. On the side opposite to the nerve section and in the spinal cords of unoperated cats, SP reaction product was densest in laminae 1 and 2. Normal staining extended across the entire width of the dorsal horn and consisted of intensely staining, coarse, clumped globules intermingled with finer granules. By 10 days post-operative SP reaction product in the L7 segment was reduced in laminae 1 and 2 on the side of nerve section. This decrease was most pronounced medially where coarse globular staining was primarily affected. Finely granular reaction product was seen in areas from which the heavier staining had disappeared. At 30 and 60 days post-operative the reduction in coarse globular staining in medial laminae 1 and 2 progressed, and the more finely granular staining appeared in its place. Ganglion cell numbers in the L7 DRG of unoperated animals showed little difference between sides; R/L ratio = 1.02 (N=3). By 60 days after right sciatic nerve transection a difference in cell numbers became apparent: the R/L ratio ranged from .87 to .81, indicating that partial deafferentation due to transganglionic degeneration must occur. Therefore, a reduction in SP staining in the partially deafferented dorsal horn may be due at least in part to ganglion cell death. A similar change in the characteristics of SP staining follows deafferentation of cat dorsal horn by complete unilateral lumbosacral rhizotomies, where an increase in finely granular staining at longer postoperative survival is most likely due to sprouting by SP containing interneurons (Tessler et al. Brain Res. 230: 263).

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84.8 ALTERATIONS OF HRP-LABELLED PUDENDAL NERVE AFFERENT PROJECTIONS IN THE SACRAL SPINAL CORD OF THE CAT DURING NEONATAL DEVELOPMENT AND AFTER SPINAL CORD TRANSECTION: CORRELATION WITH PHYSIOLOGICAL PLASTICITY OF A SPINAL SOMATOVESICAL REFLEX. K.B.Thor, D.C.Kuo, W.C.deGroat, D.Blais*, M.Backes*, Dept. of Pharmacol., Sch. of Med., Univ. of Pittsburgh, Pittsburgh, PA 15261

In adult cats activation of pudendal nerve afferents (PNA) by tactile stimulation of the perineum inhibits the micturition reflex. However, the same stimulus in neonatal kittens and chronic spinal cats initiates micturition. In the present experiments we have examined the possibility that plasticity of these somatovesical reflex mechanisms might be related to a rearrangement of afferent projections in the spinal cord. PNA terminals were labelled by transganglionic transport of HRP following its application to the central cut end of the nerve in normal and chronic spinal cats (6-8 wks. post-T12 transection) and neonatal kittens (1-5 days of age). After allowing transport of HRP, spinal cord sections were processed with TMB.

The neonatal and spinal cats showed a common PNA distribution in the sacral cord which was substantially expanded in comparison to the distribution in the normal cats. The increased labelling was most conspicuous on the side of the cord contralateral (CONTRA) to HRP application. Whereas the normal adult had sparse labelling in lateral CONTRA Lamina (LAM) I, V & VI (1 section in 20 weakly labelled), the neonatal and spinal cats had substantial labelling in those regions in almost every section, often forming a continuous ring around the CONTRA dorsal horn (DH). The CONTRA dorsal root ganglion had no cells labelled. Neonatal and spinal cats also exhibited greater labelling than normal cats in IPSI LAM I on the lateral border of the DH which extended into LAM V and VI. In neonatal and spinalized cats but not intact cats, this projection extended into LAM VII 20-30 μm ventrally along the lateral border of the intermediate grey matter. This is a region which contains bladder preganglionic neurons. A medial projection around the DH into IPSI and CONTRA medial LAM V, VI and X was heavily labelled in all animals. While the medial projection in normal animals was much stronger than the lateral projection, in neonatal and spinal cats both were heavily labelled. Those regions (i.e. lateral LAM I, V, VI & VII) which demonstrate expanded HRP-labelled PNA projections in the neonatal and spinal cats are also regions which receive excitatory pelvic nerve afferent projections from the bladder. Possibly the regression and expansion of PNA terminal fields during postnatal development and during recovery from spinal injury, respectively, represents a morphological change which underlies plasticity of somatovesical reflexes in these animals.

84.9 SPROUTING AND SYNAPSE FORMATION PRODUCED BY MARCAINE®. M. Tal and S. Rotshenker. Dept. of Anatomy and Embryology, Hebrew Univ.-Hadassah Med. Sch., Jerusalem, Israel.

Axotomy of the nerve to one cutaneous-pectoris (c.p.) muscle of the frog initiated a signal for sprouting in the injured nerve cells that transferred transneuronally across the spinal cord to intact motor neurons that innervate the contralateral intact c.p. muscle. Consequently, a polyneuronal pattern of innervation developed in the intact muscle (Rotshenker, S., J. Physiol.,292:535, 1979; Rotshenker, S. and Reichert, F., J. Comp. Neurol.,193:413, 1980). Colchicine, in concentrations that partially inhibit axonal transport also produced contralateral sprouting and synapse formation (Rotshenker, S., Neuroscience abs.10,1980). Axotomy and colchicine could initiate the signal for growth by blocking some trophic influence of a muscle derived factor on the cell bodies of the axotomized or colchicine treated neurons. To test this hypothesis, we examined whether the removal of c.p. muscle fibers, the source of the hypothetical trophic substance, will result in the development of supernumerary innervation in the opposite intact c.p. muscle. Removal of muscle fibers was obtained by the local application of the myotoxic drug Marcaine®(5% in normal Ringer for 15 min.). Degenerating muscle fibers were phagocytized leaving behind structurally intact motor axons and nerve terminals. Nerve endings that were separated from their target muscle cells were also capable of taking up HRP upon stimulation, as they do under normal conditions. Left and right c.p. muscle fibers were examined electrophysiologically for supernumerary innervation at various times after the application of Marcaine® to left muscles by recording multiple end-plate potentials. The frequency of supernumerary innervation observed in right intact muscles for the first 4 weeks after the operation was 15.6±0.8%(SEM,n=7 number of muscles examined) and thus did not differ from normal. However, 4-5 weeks after drug application the incidence of polyneuronal innervation increased and reached 31.8±2.4%(SEM,n=19). Recording of synaptic activity could also be made in left c.p. muscle fibers that did not degenerate following Marcaine® application. In such muscle fibers the incidence of polyneuronal innervation increased over normal about 3 weeks after the operation reaching 34.1±2.6% (SEM,n=19). The application of Ringer solution to left muscles or Marcaine® to their nerves at a distance from the muscle did not produce supernumerary innervation in neither left or right muscles. Our observations that the removal of left muscle fibers is followed by sprouting and synapse formation are thus consistent with the hypothesis that a muscle derived substance has a regulatory effect on the development of a signal for growth in cell bodies of motor neurons.

84.10 MOTONEURON SPROUTING CAPACITY, ENHANCEMENT BY EXOGENOUS GANGLIOSIDES. A. Gorio, P. Marini* and R. Zanoni*. Fidia Research Laboratories, Department of Cytopharmacology, 35031 Abano Terme (PD), Italy.

The soleus muscle of the rat was denervated by resecting the L_5 root. Regeneration along this route was inhibited by ligature of the proximal stump and intraperitoneal suture of the distal stump. Ten, 30 and 50 days after surgery the extent of recovery was dependent upon the number of motor units remaining in the soleus muscle and no difference was observed between the 3 groups, indicating that the limiting factor is the number of motor units remaining and not the time allowed for recovery. In this defined model we have investigated the capacity of exogenous gangliosides to stimulate sprouting. Animals were treated daily with 5 mg/kg of gangliosides, the treatment stimulated the enlargement of the remaining motor units in a highly significant manner. To quantify the extent of sprouting and stimulatory action of gangliosides, we have defined as the index of sprouting the ratio between the percentage of muscle innervation due to a certain number of motor units in reinnervation and in normal conditions. We found that when one motor unit remains in the muscle it enlarges its size up to 4.5 fold. However, if the animal is treated with gangliosides it can be enlarged up to 6.3.

Morphological analysis has shown that the most efficient type of sprout originates inside the nerve trunk. Therefore, the role of Schwann cells and nerve sheaths can be postulated for the stabilization of regenerating sprouts. These results suggest that the extent of sprouting is related to the amount of denervation. Therefore, regenerative capacity of the neuron is due to the combined action of endogenous metabolic capacities and extrinsic factors. The action of gangliosides could probably be added to this latter phenomenon. We also conclude that in nerve regeneration elongation is substained by the neuron itself, but the processes of peripheral guidance and branching are probably regulated at the periphery.

84.11 "ZAP AXOTOMY": GROWTH OF SINGLE AXONS INDUCED BY LOCALIZED FLUORESCENT DYE IRRADIATION. C. S. Cohan*, R. D. Hadley, and S. B. Kater. (SPON: F. Bahls) Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

In the pulmonate snail, *Helisoma*, axotomy results in a predictable sequence of changes in morphology and connectivity in the central nervous system. Until recently, we have studied these changes by crushing the nerves which contain the axons of identified neurons in this preparation. This report describes a technique for selective axotomy of individual neurons within intact nerve trunks. We have used this technique to determine whether the cellular changes induced by axotomy depend on damage to the surrounding environment of the growing fiber that is caused by nerve crush.

The technique we describe here was modified from studies by Miller and Selverston (*Science* 206:702, 1979) in which neurons filled with fluorescent dye were killed by photoirradiation. We penetrated the identified neuron 5 of *Helisoma* buccal ganglia and filled it with the dye, 4, 5-carboxyfluorescein. Preparations were subsequently viewed on an inverted microscope fitted with an iris diaphragm to limit the area of light exposure. Axotomy was obtained by positioning a portion of the axon within the irradiating spot (25μ in diameter) and exposing it to light of normal viewing intensity for 45 seconds using a 40 X water immersion lens (N.A. .75). To initially position the ganglia the incident light was reduced by 2 log units and viewed with a Silicon Intensified Target (SIT) camera to prevent damage to the dye filled cells (*TINS* 5:80, 1982). After zapping, ganglia were cultured in modified Leibowitz L-15 medium with co-cultured brains for a period of 5 days.

After the culture period, profuse sprouting of neuron 5 was observed. Axotomy and subsequent outgrowth was confined to the irradiated cell; other axons in the nerve were not damaged. Buccal ganglia in which both neurons 5 had been axotomized by irradiation close to the soma showed abundant sprouting in the buccal commissure and became electrically coupled to one another.

The neurite outgrowth and resultant coupling that was induced by zap axotomy was indistinguishable from that induced by nerve crushes (*Science* 212:79, 1981). From these data we conclude that: 1) irradiation of a restricted segment of a dye filled axon is an effective means of axotomizing single neurons; and 2) axotomy of an individual neuron in the absence of the massive damage to surrounding neurons and nonneuronal cells that results from nerve crushes, can induce neurite outgrowth and predictable changes in connectivity from the axotomized cell. Supported by a grant from the MDA and NS15350.

84.12 INCREASED MONOAMINERGIC INNERVATION OF SUPERFICIAL COLLICULAR LAMINAE IN NEONATALLY ENUCLEATED HAMSTERS. A. Hess[1] and R.W. Rhoades[1,2]. Dept. of Anatomy, Rutgers Medical School[1] and New Jersey School of Osteopathic Medicine[2], Piscataway, NJ 08854.

The mammalian superior colliculus is innervated by noradrenergic fibers (Swanson, L.W. and Hartman, B.K., J. Comp. Neurol., 163:467, 1975), which probably originate in the locus coeruleus (Jones, B.E. and Moore, R.Y., Brain Res., 127:23, 1977; Edwards, S.B., et al., J. Comp. Neurol., 184:309, 1979). After the crossed retinal input to the superficial collicular laminae is removed at birth, other afferents expand their terminal distributions in the partially deafferented tectum (see Rhoades, R.W. et al., J. Neurophysiol., 46:855, 1981; for citations). In the present study, histoflourescence techniques have been used to determine whether or not neonatal enucleation alters the organization of the monoaminergic (MA), presumably noradrenergic, innervation of the hamster's colliculus.

The left eye was removed from hamsters within 12 hr of birth, and they were examined no less than 4 months later. Three hrs after treatment with nialamide (200mg/kg delivered intraperitoneally), animals were sacrificed and 32μm serial, cryostat sections were cut through the colliculus and processed for the visualization of MA containing fibers by the sucrose-potassium phosphate-glyoxylic acid method (de la Torre, J.C., Neurosci. Lett., 17: 339, 1980).

In normals, and in the colliculus contralateral to the remaining eye in the neonatal enucleates, MA fibers had a bistratified distribution. Numerous fibers were visible in the upper stratum griseum superficiale (SGS) and in the stratum griseum intermediale (SGI). The density of flourescent fibers was fairly uniform throughout the rostrocaudal and mediolateral extents of the colliculus. In the tectum ipsilateral to the remaining eye, the distribution of MA fibers was also bistratified, but the superficial labelling was much denser than that in the SGI. Quantitative, within animal comparisons of the MA fiber density in the superficial layers of the two colliculi indicated a 50-100% increase on the deafferented side. This enhanced innervation was visible throughout the rostrocaudal and mediolateral extents of the colliculus. No clearcut changes were noted in the MA innervation of the SGI ipsilateral to the remaining eye in the neonatal enucleates.

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84.13 A CORRELATIVE FLUORESCENCE AND ELECTRON MICROSCOPIC STUDY OF VASCULAR SYMPATHETIC AXONS FOLLOWING SEPTAL LESIONS IN THE RAT. J. P. Chandler* and K. A. Crutcher (SPON: R. J. Mullen) Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

Lesions of the septohippocampal pathway in the rat result in sprouting of postganglionic sympathetic axons that will form the sympathohippocampal (SyH) projection. As part of a study directed at elucidating ultrastructural features of SyH fibers, we have used electron microscopy (EM) and fluorescence microscopy (FM) to identify fibers around pial and penetrating blood vessels (BV) of the dorsal dentate gyrus (DG).

Female Long-Evans rats were given electrolytic lesions of the medial septum and were killed 2-8 months later. Some also received a unilateral superior cervical ganglionectomy 3 days prior to sacrifice. Unoperated animals and animals receiving sham septal lesions and sham ganglionectomies served as controls. Detailed ultrastructural observations were made in animals that received septal lesions 2-8 months earlier.

In normal rats, occasional coarse, thick, intensely fluorescent fibers characteristic of peripheral noradrenergic axons were found around some pial BV's but not around BV's within the obliterated hippocampal fissure or around penetrating BV's in the dentate molecular layer. Following chronic septal lesions, however, numerous fluorescent fibers were associated with pial and penetrating BV's and appeared to form a plexus over the pial surface. Ganglionectomy resulted in complete ipsilateral loss of fluorescent fibers around BV's and within the DG, confirming their peripheral origin.

Although sampling is more limited by EM, vascular sympathetic fibers were encountered rarely in normal rats, and, when present, consisted of 1 or 2 axons. In animals receiving septal lesions, most extracerebral BV's were accompanied by bundles of up to 30 small (0.5-1.5 μm) diameter axons surrounded by non-neuronal cell processes and basal lamina. These fascicles were typically associated with collagen fibers. Fascicles containing up to 6 axons were directly apposed to penetrating BV's of the dentate molecular layer. Following ganglionectomy, no such fibers could be identified. At each survival time, many fascicles contained one or more profiles that contained large irregular vesicles reminiscent of those found in growth cones.

It seems possible that individual fluorescent fibers observed at the light microscopic level contain several small diameter axons at the EM level. These results suggest that vascular sympathetic fibers proliferate up to at least 8 months following a septal lesion. This study provides a basis for future EM identification of SyH fibers in the rat following septal lesions. (Supported by NIH grant #NS 17131)

84.14 EFFECTS OF SUPERIOR CERVICAL GANGLION STIMULATION ON HIPPOCAMPAL RESPONSES IN NORMAL RATS AND AFTER MEDIAL SEPTAL LESIONS. K. Krnjević, N. Ropert, J.L. Bossu*, and J. Davis, Depts. of Anaesthesia Research & Physiology, McGill University, Montréal, Québec, and Department of Medicine (Neurology) and Pharmacology, Duke University, N.C.

Destruction of septo-hippocampal fibres leads to sprouting of sympathetic fibres that originate from the superior cervical ganglion (SCG) and their growth into the hippocampus (Crutcher and Davis, Trends in Neuroscience, March 1981, p. 70). It was therefore of interest to know whether such sympathetic fibres can exert a significant effect on hippocampal electrical activity - especially in view of the marked increase in excitability of CA1 and CA3 pyramidal cells observed 3-5 weeks after septal lesions (Ropert et al. This meeting). In normal, lesioned or sham-operated rats (under urethane) the superior cervical ganglion was stimulated at intensities 1-4 times the threshold for eliciting protrusion of the ipsilateral eyeball, at frequencies of 5-10 Hz, typically for 1-2 min. Most often, there was either no change or a small depression of CA1, CA3 or dentate gyrus population spikes evoked by fimbrial or perforant path stimulation. There were also no consistent changes in the corresponding EPSP fields recorded in the stratum radiatum, or in spontaneous unit firing. The only relatively consistent effect of SCG stimulation was a potentiation of population spikes evoked by iontophoretic applications of acetylcholine (ACh). This curious action, which has a rapid time course, was particularly evident in the CA1 pyramidal layer of both normal and lesioned animals; but much less so in area CA3 and the dentate gyrus, where the facilitatory action of ACh is less readily demonstrable. Local iontophoretic applications of noradrenaline (NA) can also cause a selective enhancement of ACh-evoked population spikes and therefore to some extent support the possibility of a direct adrenergic facilitatory effect of SCG activity. However, bearing in mind the absence of any innervation from the SCG in the hippocampal parenchyma of normal rats, as well as the very moderate degree of new sympathetic innervation seen in our lesioned rats, a more likely explanation is that SCG stimulation causes local changes in blood flow which indirectly affect the action of ACh. More generally our observations do not indicate a major excitatory or inhibitory action of the sprouting SCG fibres on electrical activity of hippocampal cells; but they do not exclude a significant, long-term ("trophic") influence.

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84.15 POSSIBLE AXON-SPROUTING IN THE SUBSTANTIA NIGRA IN HALOPERIDOL-TREATED RATS. F.M. Benes*, P.A. Paskevich* and V.B. Domesick. Mailman Research Center, McLean Hospital, Belmont, MA 02178 and Depts. of Anatomy and Psychiatry, Harvard Medical School, Boston, MA 02115

The effects of haloperidol on the intrinsic organizations of brain tissue is of special importance in light of its potent effects on various aspects of CNS functions. To assess the effects of haloperidol treatment on the substantia nigra of the rat, animals (n=4) were injected with 3 mg/kg/day haloperidol IP suspended in 0.02 mM lactic acid. Controls (n=4) received lactic acid only. At the end of 16 weeks of treatment animals were perfusion-fixed with 5% glutaraldehyde - 4% formaldehyde in 0.1 M cacodylate buffer, pH 7.4. Pieces from the substantia nigra of each animal were plastic-embedded and ultra-thin silver sections of this material were subsequently viewed in the electron microscope. Samples of dendritic cross-sectional profiles from the neuropil of the transitional region between zonas compacta and reticulata in the nigral tissue from each animal were photographed at a standard magnification. Number of boutons per dendritic cross section, the volume and number of synaptic vesicles per bouton and the area of each dendritic cross section were determined for each sample (n=99). Histograms were plotted for each variable and the presence of normal vs. non-normal distributions assessed to establish whether parametric vs. non-parametric statistical analyses were indicated. The number of boutons per dendrite profile showed a non-normal distribution and was therefore subject to a Kolmogorov-Smirnov distribution analysis. This analysis showed significantly different distributions between haloperidol-treated and control animals which was a reflection of an increase of boutons per dendrite cross-sectional profile. Consistent with this increase in bouton numbers, the total bouton volume per dendrite profile was also increased. Individual bouton volume and synaptic vesicle density for each bouton appeared largely unaffected by haloperidol treatment. Although there was a trend toward a haloperidol-related increase in the mean dendrite cross-sectional area (caliber), the non-parametric Mann-Whitney rank sum test failed to show any significant difference. The results of these studies are interpreted as being consistent with axon collateral sprouting in the substantia nigra of rat in response to long-term haloperidol administration. The nerve endings involved may be those of gabanergic neurons in the neostriatum which are known to project to the substantia nigra. These results will be discussed in relation to known connection patterns, as well as various pharmacologic and behavioral correlates. This study was supported by NIMH grant PO1 MH 31154 and by NIH BRSG grant RR05484.

84.16 EFFECTS OF AGE ON SPROUTING OF DORSAL ROOT AXONS. C.E. Hulsebosch and R.E. Coggeshall. Depts. of Anatomy and of Physiology and Biophysics and the Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

Dorsal root axons are reported to sprout following either spinal hemisection or unilateral rhizotomies above and below an untouched (spared) root. To quantitate this phenomenon, we counted dorsal root axons in the electron microscope, which has the resolution necessary for visualizing both myelinated and unmyelinated axons. We found a 10-15% increase in unmyelinated axons in the roots on the operated side of rats when either type of surgery was done within the first month of life. We interpreted this increase in axonal number to be axonal sprouting.

The present study is concerned with the effect of age on this process. We asked first if the axonal increase changes or remains the same as the hemisected rats age, and second, if the axonal increase is dependent on the age of the rat when the hemisection is done. For the first question, we find that the 10-15% increase persists as the rats age. For the second question, we find no increase in unmyelinated axons if the operation is done in a rat that is 1 year old. Thus if further work confirms these preliminary data, we conclude that the axonal increase which occurs in young animals does not change markedly as the animals age, and that there is no axonal increase if the surgical procedures are done on older animals.

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84.17 MOTOR AXON SPROUTING PATTERNS CORRELATE WITH ENDPLATE TYPE IN THE TIBIALIS ANTERIOR MUSCLES OF THE RAT. Riley, D.A. and C.S. Fahlman*. Department of Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

Sprouting of axons in 200-230g rats was induced by unilateral application of colchicine to the sciatic nerve at a concentration (5 mM) that produced no detectable destruction of intramuscular nerves. Ten days following drug exposure, tissue sections were processed using a combined silver-cholinesterase technique to stain motor axons and localize endplates. Three morphologically distinct types of endplates were recognized in both normal and experimental muscles: A type C (complex), the largest of the junctions, possessed an axon that terminated in multiple long and slender processes with numerous secondary branches. The smallest junction, type S (simple), was characterized by a single short and thick terminal process that rarely bore side branches. The intermediate (I) junction had an axon with multiple long and thick terminal processes that evinced a few stubby secondary branches. Sampling 2000-3000 endplates per muscle revealed low levels of sprouting in normal muscles: internodal (1.31%), ultraterminal (0.75%) and preterminal (0.06%). This sprouting is consistent with descriptions by previous workers of endplate elaboration during aging. Sham treated preparations and muscles contralateral to the drug-treated side were not significantly different from normal. Colchicine caused type C endplates to produce mostly internodal (7.10%) and some ultraterminal (2.37%) and preterminal (0.62%) sprouts. Type I junctions exhibited equivalent amounts of internodal (4.20%) and ultraterminal (4.39%) sprouting, but little preterminal sprouting (0.49%). Type S endplates sprouted the least frequently with somewhat more ultraterminal (2.75%) than internodal (1.43%) sprouting and substantial preterminal (0.69%) sprouting. The variations in sprouting patterns among different types of endplates correlated with the preexisting degree and type of terminal branching. Axons ending in multiple long and highly branched arbors responded to the sprouting stimulus by producing mostly internodal sprouts which have the capacity to branch extensively. Conversely, the least branched axons found at type S junctions responded more often by elongating the existing terminal axons than producing additional terminal branches. Supported by NIH grant 5R01 NS18110-02.

84.18 FAILURE OF THE HIPPOCAMPAL ASSOCIATIONAL AFFERENTS TO OCCUPY THE ENTIRE MOLECULAR LAYER OF THE DENTATE GYRUS AFTER COMBINED ENTORHINAL AND FIMBRIAL LESIONS. G.M. Peterson. The Salk Institute, La Jolla, CA 92037.

The major extrinsic afferents to the rat dentate gyrus show a remarkable capacity for sprouting when one or more of the contiguous fiber systems is removed. For example, after removal of the entorhinal afferents in young rats (before postnatal day 14) the associational fibers, which normally terminate within the inner one-third of the molecular layer, spread out over the entire width of the molecular layer; after the same deafferentation in adult animals the fibers are still capable of sprouting but increase their terminal projection field by only 40%. It has been suggested that one factor limiting the spread of the associational fibers in adult animals is the concurrent reactive sprouting of the septal afferents within the outer half of the molecular layer. The septal afferents sprout only slightly after an entorhinal lesion in the young rat, but sprout to occupy the outer half of the molecular layer in adults. This reciprocity between the sprouted associational and septal projections and the fact that the two sprouted systems occupy adjacent, but non-overlapping zones suggest that these two afferent systems may be in competition with each other during reactive sprouting.

To test whether the limited spread of the associational fibers after entorhinal lesions in adult animals is due to an interaction with the simultaneously sprouting septal afferents, a series of adult rats has been prepared in which most of the extrinsic afferents to the dentate gyrus have been eliminated. To do this the entorhinal cortex was ablated bilaterally and at the same time the ipsilateral fimbria was transected near the rostral pole of the hippocampus. Eight weeks later the distribution of the associational afferents was determined in preparations stained by Timm's sulphide silver method and in autoradiographs following the injection of ^{3}H-proline into the hilar region of the dentate gyrus at caudal levels. The measurements of the width of the expanded associational zones in Timm stained material and grain density profiles in the autoradiographs clearly indicate that even when both lesions were complete (as confirmed histologically) the associational afferents do not extend into the outer third of the molecular layer. It would appear, therefore, that the sprouting of the septal afferents is not by itself the critical factor limiting the outward spread of the associational afferents. Whether some other extrinsic agent (such as a glial hypertrophy or the sprouting of intrinsic afferents) is involved, remains to be determined. Alternatively, the combined effects of sprouting to replace the entorhinal input to the dentate gyrus, and the pruning effect induced by interrupting the commissural collaterals, may effectively exhaust the capacity of the associational system to sprout beyond the inner half of the molecular layer after entorhinal cortex ablation.

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85.1 FIXATION OF SPINAL REFLEX ALTERATIONS WITH DIRECT STIMULATION OF THE RAT TIBIAL NERVE. J. E. Steinmetz* and M. M. Patterson. College of Osteopathic Medicine, Ohio Univ., Athens, OH 45701.

We have recently demonstrated that long-lasting hindlimb flexion could be induced by 30 min of stimulation delivered to the thigh-skin of spinalized rats. Moreover, several studies have suggested that these postural changes are initiated and maintained by neural alterations that occur at the spinal level. This phenomenon has been referred to as "spinal fixation". The present study was conducted to demonstrate the fixation phenomenon using direct sensory nerve stimulation to induce muscle contraction. Rats were first anesthetized with Nembutal, spinalized at T-7, and the left tibial nerve and tarsal tendons of the peroneus longus and tibialis cranialis muscles dissected free. After securely clamping the leg, the proximal end of the cut tibial nerve was tied to a bipolar electrode and the flexor muscle tendons attached to a force transducer which monitored muscle contraction via a polygraph. Twenty-eight animals were randomly assigned to four groups that received either 40, 30, 20, or 10 min of stimulation (2-4 uA, 80 pps, 2 msec) through the bipolar electrode. At the end of the stimulation period, the stimulation was terminated and muscle contraction monitored for an additional 5 min. Subsequent to the poststimulation period, the nerve innervating the tarsal flexors (n. fibularis) was severed and loss of muscle tension observed. Statistical analysis revealed no differences in muscular force recorded during the four stimulation periods. Significant differences were discernible, however, when poststimulation contraction was analyzed. Poststimulation period contraction was directly proportional to stimulation period length. Additionally, severing neural input to the muscle resulted in greater losses of contraction as stimulation time increased. Data from two control groups revealed that poststimulation tension was not a result of stimulation arising from the previously stimulated tibial nerve and could not be obtained when flexor muscle contraction was induced by stimulation of the fibularis motor nerve.

85.2 IMPAIRED DRL PERFORMANCE WITH ELECTROLYTIC MEDIAN RAPHE LESIONS. L. L. Wing* and D. Wirtshafter (SPON: J.D. Davis). Dept. of Psych. University of Illinois at Chicago, Chicago, IL 60680.

In previous studies we and other workers have demonstrated that electrolytic lesions of the median raphe nucleus produce a number of behavioral effects resembling those seen after damage to limbic structures such as the hippocampus and septum. For example, median raphe lesions increase open field activity, slow extinction in the straight alley, retard the reversal of position habits in a T-maze, eliminate spontaneous alternation, impair performance on an 8-arm radial maze task, and eliminate latent inhibition in a shuttle task. These results are in agreement with anatomical data suggesting a close functional relation between limbic structures and the paramedian midbrain tegmentum.

In order to further investigate the similarities between the effects of limbic and median raphe damage we examined the effects of electrolytic median raphe lesions on the acquisition of a differential reinforcement for low rates of responding (DRL) schedule. DRL acquisition is known to be severely disrupted by hippocampal and septal lesions. Rats received 9 daily 20-minute sessions of continuous reinforcement (CRF) in an operant box followed by 24 days on a DRL-20 second schedule. Median raphe lesions did not effect lever pressing under CRF, but impaired performance under the DRL condition. Animals with lesions lever pressed at significantly higher rates and received significantly fewer reinforcements than did sham-operated controls.

Recent evidence has suggested that many of the effects of electrolytic lesions of the raphe are not secondary to serotonin depletion. In order to investigate the role of serotonergic mechanisms we are examining DRL performance following injections of the serotonin neurotoxin 5,7-dihydroxytryptomine (5,7-DHT) (7.5 µg in 1.5 µl) into the median raphe nucleus of desmethylimiprimine pretreated rats. Results to date suggest that 5,7-DHT injections do not alter performance relative to that of vehicle injected animals. Current findings provide further support for the concept of a functionally important limbic-midbrain circuit. In agreement with other reports, however, the present study also suggests many of the behavioral effects of electrolytic median raphe lesions are not secondary to serotonin depletion, but may reflect damage to fibers of passage or non-serotonergic neurons originating in the raphe.

85.3 FAILURE TO REPLICATE THE DORSAL BUNDLE EXTINCTION EFFECT: TELENCEPHALIC NOREPINEPHRINE DEPLETION DOES NOT RELIABLY INCREASE RESISTANCE TO EXTINCTION BUT DOES AUGMENT GUSTATORY NEOPHOBIA. T. N. Tombaugh, B. A. Pappas, D. C. S. Roberts, G. J. Vickers*, and C. Szostak*. Psychology Dept., Unit for Behavioral Medicine and Pharmacology, Carleton Univ., Ottawa, Ont. K1S 5B6.

Depletion of telencephalic noradrenaline (Ne), caused by lesion of the dorsal tegmental bundle, has been reported to increase persistence of nonreinforced responding in various operant tasks. This has been referred to as the dorsal bundle extinction effect (DBEE). In an effort to reproduce this effect, rats receiving 6-hydroxydopamine lesions of the dorsal Ne bundle (DB-6OHDA) were compared to controls during extinction of a continuous food rewarded (CRF) lever-press response. While the lesioned group showed an increase in responding during the first two minutes of the extinction test, no differences were found between the groups on any measure of persistence of nonreinforced responding. Moreover, no differences in reinforced response rates were observed with CRF, fixed ratio (FR-15, FR-30, FR-60) or variable interval (VI-30, VI-60, VI-120 sec) schedules of reinforcement. In order to test the hypothesis that the DBEE is dependent on time of behavioral testing after surgery, subsequent experiments were performed where rats began CRF operant training 5, 17, 31 or 110 days post-lesion. No differences in resistance to extinction were observed between lesion and control rats at any post-lesion interval. Neonatal treatment with 6-OHDA which permanently lesions forebrain Ne terminals also failed to prolong extinction. Finally, when both DB-6OHDA and neonatal rats were given a choice between water and saccharine the lesioned animals exhibited a neophobic reaction whereby they drank significantly less saccharine. We conclude that while the DBEE is not a reliably reproducible phenomenon other effects of forebrain Ne lesions, such as neophobia, appear to be robust.

85.4 AREA POSTREMA LESIONS IN RATS ENHANCE THE MAGNITUDE OF BODY-ROTATION INDUCED TASTE AVERSIONS. Klaus-Peter Ossenkopp. Dept. of Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5C2

Area postrema (AP), a circumventricular organ located at the caudal end of the fourth ventricle on the dorsal medulla, has been shown to be a chemoreceptive area involved in emesis (Borison, Life Sci., 1974, 14, 1807) and in taste aversion (TA) learning induced by certain pharmacological agents (e.g., Ritter et al., Brain Res., 1980, 201, 501). Destruction of AP strongly attentuates vomiting frequency in dogs and monkeys subjected to body motion which normally induces a strong emetic response. However, little is known about the role of AP in TAs induced by body rotation. Rats readily acquire an aversion to a novel taste paired with exposure to body rotation. In the present experiment a novel 0.1% sodium saccharin solution was paired with 30 min of body rotation (70 rpm; schedule of 15 sec on, 5 sec off), followed the next day with a two-bottle choice test (saccharin vs water). Three blocks of a conditioning trial followed by a test trial were given to rats with AP lesions, sham lesions, or no lesions. A fourth group of rats (no lesions) was given the novel saccharin taste followed by a sham rotation procedure. The group given the sham rotation exhibited an increasing preference for, and intake of, the saccharin solution. All the other groups displayed an increasing aversion to the saccharin taste with the AP lesioned group showing a significantly greater decrease in saccharin consumption relative to the sham lesion and no lesion groups. Thus, AP lesions enhanced the TA induced by the body rotation treatment.

Subsequent TA conditioning involving chocolate milk paired with injections of scopolamine methyl nitrate (SMN) i.p. (1 mg/kg) resulted in strong TAs to the chocolate milk for all groups except the AP lesioned group. Rats with ablated AP exhibited increased consumption of chocolate milk over conditioning days. The integrity of AP is necessary for TA conditioning with SMN but not with body rotation. The enhanced aversion found in AP lesioned rats subjected to body rotation, may be the result of an increased hedonic value of the saccharin taste in these animals resulting in a stronger conditioning effect (cf. Edwards et al., Brain Res., 1981, 216, 265).

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85.5 BASAL AND CENTRAL AMYGDALA INVOLVEMENT IN THE ACQUISITION OF TASTE AND ODOR AVERSIONS. F. Bermudez-Rattoni*, S.W. Kiefer, C.V. Grijalva, and J. Garcia* (SPON: M. Schlag-Rey). Dept. Psychol., Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Normal rats acquire aversions to each component of an odor-taste compound when it is followed by illness. The odor aversion is greater following compound odor-taste conditioning than when odor alone is paired with illness (a phenomena called potentiation). In this experiment the role of nuclei in the amygdala complex was tested in the conditioning of aversions to taste, odor, or an odor-taste compound. Rats were given small electrolytic lesions in the basal amygdala including the lateral and medial nuclei (BA, n=18), central amygdala nucleus (CA, n=18), or sham operations (Sh, n=18). Following postoperative recovery each group was divided into three subgroups. Aversion conditioning to either taste alone, odor alone, or the odor-taste compound was done by following the stimulus presentation with delayed illness. Almond odor and .1% saccharin were the conditioned stimuli while the unconditioned stimulus was 190 mg/kg lithium chloride ig.

After conditioning, tests with odor alone showed that rats which received the compound odor-taste developed the strongest odor aversions (consumption reduced to 15% of water baseline). Rats which received taste-illness conditioning did not show an odor aversion (90% of water baseline) while the animals which had odor-illness training reduced consumption to about 60% of water baseline. Thus, rats with basal or central amygdala lesions developed normal odor aversions; in addition, the rats with lesions showed normal potentiated odor aversions. When tested with the taste cue, all rats given odor-illness training were at water baseline. In contrast, normal and BA rats which received taste-illness training developed strong saccharin aversions (9% of water baseline). Rats with central amygdala lesions developed significant aversions but these were not as strong as in the other taste-ill groups (consumption was at 30% of water baseline). The same pattern of results was found in rats given odor-taste conditioning: CA rats did not display as strong a taste aversion (52% of water baseline) as the normal and BA rats (18% of water baseline). These preliminary data suggest that the central nucleus of the amygdala is involved in the formation of taste-illness associations but not in the taste potentiation of odor aversions in delayed poison conditioning.

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85.6 MEMORY IMPAIRMENT IN ADULT RATS DUE TO MILD OR SEVERE ZINC DEFICIENCY AND/OR UNDERNUTRITION DURING GESTATION AND LACTATION. E.S. Halas, J.B. Campbell* and H.H. Sandstead*. Psychology Dept. and Anatomy Dept., Univ. of North Dakota and USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202.

Two experiments studied the effects of mild zinc deficiency (10 ppm Zn) and/or undernutrition during gestation and lactation on short-term memory (STM) and long-term memory (LTM). A third experiment studied the effects of severe zinc deficiency (0 ppm Zn) during lactation on STM and LTM. Each experiment had three groups: a zinc deficient group (ZD), a pair fed undernourished group (PF), and a normal control ad lib fed group (AL). There were 8 rats in each group for all 3 experiments.

Using a 17 arm radial maze, we found that first generation ZD rats were significantly inferior in STM when compared to PF and AL rats. There were no significant differences between the PF and AL rats. The second generation rats were not tested. For the third generation of rats, the ZD and PF were significantly inferior in STM when compared to the AL rats. There were no significant differences between the ZD and PF rats. The results suggest that mild zinc deficiency impairs STM in the first generation whereas mild undernutrition (PF) requires more than one generation before it shows any adverse effects on STM.

In another experiment, we observed the effects of mild zinc deficiency on LTM as well as on STM. LTM can be studied by baiting only 8 out of 17 arms in the radial maze. Arms 1, 2, 6, 9, 10, 11, 14 and 16 were baited every day while the other arms were never baited. The rats were given 1 trial per day for 40 days. To be optimally successful, the rat should enter each baited arm once every day and avoid the unbaited arms. Avoiding the unbaited arms is a test of LTM whereas the baited arms are a test of STM. Our results clearly indicate that first generation ZD rats were significantly inferior in LTM when compared to PF and AL rats. The PF rats were significantly inferior to the AL rats. Over the 40 trials, our results also suggest the ZD rats were significantly inferior to PF and AL rats in learning the radial maze. Although the ZD rats did not perform as well as the PF and AL rats on the STM portion of the maze, the results were not significantly different.

The failure of the ZD rats on the LTM test of the radial maze provides confirmation of previously published data using an entirely different experimental procedure to test LTM. It is also noteworthy that mild zinc deficiency extended over the gestation and lactation periods can be as disruptive of LTM as severe zinc deficiency during the lactation period. LTM was not affected when severe zinc deficiency was imposed during the third trimester of pregnancy.

85.7 TRIMETHYLTIN INDUCED CHANGES IN ^{3}H-QNB BINDING IN VARIOUS RODENT BRAIN AREAS. P.R. Sumner* and J.D. Hirsch (SPON: R.L. Kochman). Dept. Biol. Res., G.D. Searle & Co., Chicago, IL 60680.

Trimethyltin (TMT) is a neurotoxin which produces structural alterations in neurons of rat hippocampus and other brain regions. (Brown, et al., Am. J. Path., 1979) Recent work indicates that this compound may be useful in producing functional deficits in long term memory and learning in rodents. Also, cholinergic neurons of the limbic system are thought to be important for learning and memory. We therefore wanted to investigate the effects of TMT-hydroxide (TMT-OH) on muscarinic cholinergic receptor binding in the hippocampus and other brain regions of rodents.

Male Charles River rats and mice were used in these studies. Animals were injected with TMT-OH in 0.9% saline at doses of 1.75 to 7 mg/kg, i.p., and were sacrificed at various times after drug administration. Control animals received an equal volume of 0.9% saline. The following brain regions were dissected and frozen at -80°C until assayed: hippocampus, amygdala, frontal cortex, cerebellum, hypothalamus, striatum, and olfactory bulb. ^{3}H-QNB (2.0 nM, final concentration) binding was assayed in 50mM Tris-HCl buffer, pH 7.4 at 37°C for 45 min using 10^{-4}M atropine sulfate to determine non-specific binding. Assays were terminated by filtration.

1) In mice injected with 2.5 mg/kg TMT-OH there was an 18% decrease in ^{3}H-QNB binding in the amygdala ($p<.005$) and a 24% decrease in the frontal cortex ($p<.025$) 19 days after treatment. There were no significant changes in the other regions. A dose of 1.75 mg/kg of TMT-OH had no effect on ^{3}H-QNB binding. 2) In rats sacrificed 3.5 weeks after a single 7 mg/kg injection of TMT-OH, there was a 15% decrease in binding in the amygdala ($p<.025$) and an 18% decrease in the striatum ($p<.05$). There were no significant changes in other regions. 3) In mice given two 2.5 mg/kg TMT-OH injections 4 and 7 weeks before sacrifice, there was an 11% decrease in ^{3}H-QNB binding in the amygdala. 4) In a time-course experiment mice were given a single injection of 2.5 mg/kg and groups were sacrificed 6 hr, 1, 2, 4, 7 and 14 days later. In the hippocampus there was a significant ($p<.05$) 10% decrease in ^{3}H-QNB binding only at day 4, compared to controls. These results suggest that this dosage of TMT produces transient damage to hippocampal muscarinic receptors.

In summary, there were only small, long-term changes in ^{3}H-QNB binding in response to TMT-OH treatment of rodents. These decreases were evident mainly in the amygdala. Further work is required to determine if TMT treatment will provide a useful biochemical model that correlates with memory and learning deficits.

85.8 POSTERODORSAL SEPTAL LESIONS IMPAIR RETENTION OF WIN-SHIFT AND WIN-STAY LEARNING IN A T-MAZE. M.E. Stanton*, G.J. Thomas, and G.N.O. Brito. Center for Brain Research, University of Rochester Medical Center, Rochester, NY 14642.

Claims of septo-hippocampal involvement in cognitive maps or working memory have relied heavily on support from lesion studies employing the 8-arm-maze and T-maze. In these tasks, rats are rewarded only when they avoid previously visited locations, a response requirement that makes use of the rat's spontaneous alternation tendency. Septal damage disrupts spontaneous alternation itself and, in the 8-arm-maze, induces a reliable tendency to repeat, rather than avoid, previous choices. The present study examined the effects of septal lesions on Win-Shift and Win-Stay problems in a T-maze. We reasoned that disruption of an alternation tendency by the lesion would lead to an impairment only on the Win-Shift problem whereas disruption of spatial memory would produce impairments on both problems.

Two groups of rats were trained preoperatively with a "paired-run" procedure. A trial consisted of two consecutive runs: an "information run", where the rat was forced either left or right, followed by a "choice run", where the rat could enter either arm. The Win-Shift group was reinforced only for entry into the opposite arm, whereas the Win-Stay group was rewarded only for entry into the same arm, as was entered on the information run. The direction of consecutive information runs followed an irregular schedule.

During initial preoperative training (inter-run interval, IRI=0 sec), the Win-Shift group achieved a median of 100% correct earlier than the Win-Stay group. When the IRI was subsequently increased to 30, 60, 90, and 210 sec, the choice accuracy of both groups declined significantly. This indicates that these 2 tasks depend on short-term memory and that choice accuracy was indeed influenced by the information run. Following additional training with the 0-sec IRI, subjects in both groups received stereotaxically guided, electrolytic lesions in either posterodorsal septum or neocortex. In both the Win-Stay and Win-Shift conditions, subjects with septal lesions (but not controls) showed a marked postoperative retention deficit which was ameliorated with additional training but accuracy did not return to preoperative levels.

These results confirm other reports of deficits in retention of T-maze alternation following damage to the septum or hippocampus but further show that spatial memory deficits are not confined to problems which make use of the rat's spontaneous alternation tendency.

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85.9 MEDIAL SEPTAL LESIONS, RADIAL ARM MAZE PERFORMANCE, AND SYMPATHETIC SPROUTING: A STUDY OF RECOVERY OF FUNCTION. R. P. Kesner and K. A. Crutcher. Dept. of Psychology and Dept. of Anatomy, University of Utah, Salt Lake City, UT 84112.

It has been shown that lesions of the medial septum result not only in depletion of AChE in the hippocampal formation, but also in sprouting of peripheral sympathetic fibers from the superior cervical ganglion into the dentate and hippocampal gyri. The purpose of the study was to determine whether this sprouting response has functional significance. Male Long-Evans rats were given ten pretraining trials on a radial arm maze. The animals then received medial septal lesions or sham operations. Starting the day after surgery the rats were tested daily for performance on the radial arm maze. Changes in water intake were monitored daily and activity level was measured in an open field before and after surgery. Animals receiving septal lesions displayed an initial deficit on the radial arm maze (large number of errors) followed by recovery with a time course comparable to the sympathetic sprouting response. Twenty-five days after surgery half of the animals in each group received either bilateral removal of the superior cervical ganglion or a sham operation. Removal of the sympathetic innervation with superior cervical ganglionectomies had no effect on recovery of radial arm maze performance. No critical changes occurred in activity level and no recovery was seen in lesion-induced increases in water intake.

There was a significant relationship between behavioral recovery and the degree of hippocampal AChE depletion ($r = .79$). It is concluded that recovery of radial arm maze performance is not mediated or inhibited by sympathetic sprouting following septal lesions, but might be mediated by residual septo-hippocampal fibers.

85.10 DISRUPTION AND RECOVERY OF HIPPOCAMPAL SPATIAL BEHAVIOR AFTER MEDIAL SEPTAL LESIONS IS NOT INFLUENCED BY SYMPATHETIC GANGLIONECTOMY. L. E. Harrell*, T. S. Barlow*, and James N. Davis (SPON: B. Crain). Dept. of Medicine (Neurology/Gerontology), Veterans Administration Medical Center and Duke University, Durham, N.C. 27705.

Cholinergic denervation of the rat hippocampus by lesions of the medial septal nucleus or fornix leads to the ingrowth of sympathetic fibers from the superior cervical ganglion. The importance of the hippocampus in spatial and memory behaviors led us to study the functional significance of this sympathetic ingrowth by measuring the performance of rats on a radial eight arm maze.

Adult male Sprague-Dawley rats were trained to approach 4 baited arms and ignore 4 unbaited arms of a standard radial eight arm maze. Daily testing was performed with one trial per day until criterion was achieved (selection of 4 baited arms out of the first 5 arm choices over 7 consecutive days). Twelve rats reached this criterion by 18-25 days and then underwent either medial septal lesions or sham operations. After surgical procedures testing was continued until preoperative criterion was again achieved. All rats then underwent bilateral superior cervical ganglionectomy followed by behavioral testing until criterion was reached a third time.

Medial septal lesions markedly disrupted maze performance in all animals. Initially, lesioned animals selected more arms with increased errors, defined as entering either unbaited arms or re-entering baited arms. With continued testing both response to unbaited and later response to baited arms returned to pre-operative levels. Sham operation had no disrupting effect on maze performance. Return to preoperative criterion in the lesioned animals occurred in approximately forty days. Subsequent superior cervical ganglionectomy had no effect on maze performance.

These data demonstrate that 1) the projection from the medial septum to the hippocampus is important in spatial learning, 2) a recovery of this radial arm maze behavior occurs after septal lesions, 3) the recovery of response to baited and unbaited arms has a different time course suggesting different recovery mechanisms could be involved in these behaviors, and 4) sympathetic ingrowth does not play a role in the recovery of function in this specific behavior.

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85.11 HIPPOCAMPAL FUNCTION AND THE MEMORY FOR SERIAL PATTERNS, Matthew L. Shapiro*, Stewart H. Hulse*, and David S. Olton. Department of Psychology, Johns Hopkins University, Baltimore, Maryland 21218.

Human amnesics process information contained in serial patterns differently than information not so organized. The present experiment was designed to determine if remembering elements within a serial pattern required the same memory processes as those same elements when not in a pattern. Rats were trained to run to each of the four arms of a radial maze in a sequence determined by food quantity. At the end of each arm of the maze were placed either 18, 6, 1, or 0 sucrose pellets. Rats learned to enter arms in order: 18-6-1. After performing consistently, each rat was given either a control operation, a lesion of the amygdala, or a lesion of the fimbria-fornix (FFx). Rats with control operations or amygdaloid lesions performed consistently well post-operatively. After rats with FFx lesions had recovered, they also showed accurate performance on the 18-6-1 sequence. However, when forced to enter the arm with 6 pellets first, rats with FFx lesions entered the arm with 18 pellets, returned to the arm with 6 pellets, and then entered the arm with 1 pellet. In contrast, rats with control operations or amygdaloid lesions did not return to the arm with 6 pellets after being forced to that arm initially, but entered the arm with 18 pellets and then the arm with 1 pellet.

These results indicate that memory systems responsible for processing information about elements within serial patterns differ from those that process information out of such sequences, and are related to similar findings in human amnesics. The results are discussed in terms of the differences between flexible, working memory systems that process information involving temporal/personal context regardless of its organization, and reference memory systems that process information that is organized into rule specified sequences.

85.12 THE EFFECTS OF HIPPOCAMPAL LESIONS IN RATS ON THE LEARNING OF A SEQUENCE OF INTRA-MAZE STIMULI. Donna J. Hughey*and Richard J. Koppenaal* (SPON: Samuel M. Feldman). New York University, Dept. of Psychology, New York, N.Y., 10003.

In order to study the nature of expectancies which may arise in rats during maze learning,this experiment compared the behavior of normal and hippocampal lesioned rats when they were confronted with the disconfirmation of a familiar sequence of intra-maze stimuli.

Two mazes were used. Various patterns of visual and tactile cues made the passageways of each maze unique. With the exception of the start boxes (SBs), however, the mazes had identical floor plans. In addition, the SBs were detachable and interchangeable.

Male, food-deprived, hooded rats received post-operative training in both mazes over 18-22 days. Both groups learned to run the mazes at approximately equal rates. All were running quickly and with virtually no errors by the end of this time.

The subsequent two-day test phase was procedurally identical to training, except that the SBs were interchanged, thereby reversing the SB-maze associations which had remained constant during training. No difference was seen between the training and test data of the lesioned animals. The controls, however, showed a profound disruption of goal-directed behavior upon discovering the SB switch. This novel sequence of familiar stimuli elicited exploration and apparent confusion in the normals, while the hippocampal lesioned animals were unperturbed by the change.

The behavior of the control rats seems to verify that they come to remember each SB as the first link in a unique chain of stimuli, and they develop the appropriate expectancies about the sequential arrangement of these maze cues. The performance of the lesioned animals is less easily explained, albeit consistent with several theories of hippocampal function. Although the precise mechanism of this lesion-induced deficit is not yet clear, there does appear to be some fundamental disruption in the ability to fully, or at least normally, utilize the sequential organization of environmental cues.

85.13 SEX DIFFERENCES IN THE EFFECTS OF UNILATERAL HIPPOCAMPAL LESIONS ON SPATIAL LEARNING. Barbara A. Therrien*, Dianne M. Camp* and Terry E. Robinson (SPON: H. Buchtel). School of Nursing, Psychology Department and Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

In human and non-human animals bilateral damage to the hippocampal formation results in a profound impairment in spatial learning. However, in humans the deficits in spatial learning are thought to be due primarily to right, and not left hippocampal (HPC) damage. This study was conducted to determine if left vs right HPC lesions have differential effects on spatial learning in rats. Since sex differences have been reported in brain laterality in humans and in rats we tested both males and females. Adult male and female Holtzman rats were given right (RM,RF), left (LM,LF) or bilateral (BM,BF) electrolytic HPC lesions and allowed 2 weeks to recover. They were then trained on the spatial learning task described by Morris (Lrn. & Motiv.,12(1981)239). This involved learning to escape from a 1.5 m diameter white pool filled with 20°C opaque water by swimming to a platform concealed 1 cm under the water surface. Each animal was given 4 trials/day. A trial consisted of placing the rat randomly in one of 4 locations and recording (a) the latency to reach the goal, and (b) the initial direction the rat swam. From this, the angle error was calculated. (Angle error = the angle between the initial swimming direction and the goal.) The animals were given 4 days of training with the goal in a fixed location (initial training), before it was moved to a new location (1st retraining). After 4 more days of testing it was moved once again (2nd retraining). There was no difference between male and female sham-operated controls on this task. However, compared to controls LF, LM and RM groups showed marked deficits in the time taken to reach the goal during the initial training and both retraining periods ($p<.05$), and in angle error for the 2 retraining periods ($p<.001$). In contrast, the RF's did not differ from controls in their latency to reach the goal during the first 2 training periods but did differ during the 2nd retraining period. During the 1st retraining period LF's took significantly longer to locate the goal than RF's ($p<.05$), although the two groups had equally large angle errors. The two unilaterally lesioned male groups did not differ at any time. In males unilateral lesions produced as large a deficit in angle error as did bilateral lesions, while in females bilateral lesions resulted in greater deficits than unilateral lesions. We conclude that at least in female rats there is a functional asymmetry in the organization of the hippocampal system. In addition, the differences in the effects of both unilateral and bilateral lesions in males vs females suggest that the hippocampus may be a sexually dimorphic structure.
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85.14 COLCHICINE INJECTIONS OF RAT HIPPOCAMPUS RESULT IN LEARNING DEFICITS. D.A. Stein*, E.W. Lothman and A.W. Toga (SPON: E. Montgomery). Dept. of Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110.

An intact limbic system is necessary for the performance of various learning and memory tasks. In the present experiments, the effects of lesioning one neuronal element of the hippocampal complex on learning behavior was studied. Discrete lesions of the granule cell layer of the dentate gyrus enabled us to determine if these cells are important in learning. Granule cells were selectively destroyed with four stereotactic microinjections of colchicine, 4.0 nmol in 0.2µl of normal saline, pH 7.2-7.4, through a 32 gauge cannula. Subsequent histological examinations confirmed the effectiveness of lesions (Goldschmitt and Steward, PNAS 77:3041, '80). Three groups of animals were studied behaviorally; those injected with colchicine, those receiving saline injections into the same areas, and non-operated controls. Subjects recovered from surgery for 30 days and were then food deprived until they reached approximately 80% of their free feeding weight. Training and testing took place in an operant chamber designed to deliver food pellets contingent upon the appropriate illumination of cue lamps and the required number of bar presses. Animals were trained to bar press for food on a fixed ratio. When stable responses were achieved a S^+ (blue cue lamp), S^- (red cue lamp) discrimination was imposed. Food could only be obtained during S^+. Gradually a delay of the occurrence of S^- was introduced for pressing during S^-. If animals reached criterion performance (two consecutive days at 90%), the ratio became variable. Following criterion performance during this phase, the positions of S^+ and S^- were reversed.

Colchicine animals demonstrated consistently poorer performance than either of the two control groups. None of the colchicine animals achieved criterion performance of the cue reversal task although trained twice as long as controls. Some of the colchicine rats were even unable to perform with the variable ratio task. This suggests that the animals were not attending to the S^+ cue but rather testing the outcome of bar pressing during both S^+ and S^-. All control subjects were able to perform at criterion in all of the training/testing phases.

These experiments suggest that the granule cells of the dentate gyrus are a critical component to those mechanisms necessary for learning. Since the training and testing was not begun until 30 days post surgery the results cannot be attributed to the trauma of injection. Also the training/testing was continued up to 120 days after the injection which points to the permanence of the deficit.

85.15 DEFICITS IN SPATIAL LEARNING ABILITY FOLLOWING NEONATAL MEDIAL PREFRONTAL/CAUDATE NUCLEUS LESIONS. J. P. Vicedomini,* W. Isaac,* D. Lieber,* and A. J. Nonneman. Dept. of Psychology, University of Kentucky, Lexington, Kentucky 40506-00442.

In previous studies we have shown that removal of the medial prefrontal (MF) cortex in adult rats produces behavioral impairments on spatial tasks (spatial reversal or alternation learning); while, comparable damage incurred during infancy does not compromise performance on these same tasks (Kolb, B. and A. J. Nonneman, Brain Res. 151: 135, 1978; Nonneman, A. J. and J. V. Corwin, J. Comp. Physiol. Psychol. 95: 588, 1981). Furthermore, it has been shown that cortical zones adjacent to MF cortex need not be left intact to observe this functional sparing (Vicedomini, J. P., J. V. Corwin, and A. J. Nonneman, Physiol. and Behav., in press).

The integrity of subcortical areas related to prefrontal cortex, the caudate nucleus (CN) and mediodorsal thalamus (MD), may be important to the functional sparing observed in neonatal MF operates. Lesions to either of these subcortical structures in adult or juvenile subjects produce impairments on spatial tasks, while, similar lesions produced in neonates leave spatial learning ability intact (Vicedomini, J. P., J. V. Corwin, and A. J. Nonneman, Physiol. Psychol., in press). Perhaps in this case the absence of deficits in neonatal CN or MD operates reflects recovery mechanisms dependent upon the undamaged prefrontal cortex.

The present study examined this latter hypothesis by assessing spatial learning ability in rats given combined MF plus CN lesions during infancy. Two different behavioral tasks were used, T-maze spatial alternation and place learning/reversal tests in a Morris water tank. Both tasks differentiate between the performance of neonatal and adult subjects with MF cortex damage. In comparisons with sham operated age/litter matched controls, subjects given combined MF/CN lesions were severely retarded on both spatial learning tasks.

85.16 ON THE CONTRIBUTIONS OF CORTICAL AND SUBCORTICAL SYSTEMS TO THE ACQUISITION AND MAINTENANCE OF SPATIAL LOCALIZATION STRATEGIES. R. J. Sutherland, B. Kolb and I. Q. Whishaw. Dept. of Psychology, The Univ. of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

Damage to many brain structures disrupts the ability to orient in space. However, the contributions of these various structures must be very different. Using a rat model, we have examined spatial orientation after damage to the hippocampal formation, frontal cortex, posterior cortex, ventral mesencephalic tegmentum (electrolytic or 6-OHDA), hemidecortication, or bilateral enucleation. An attempt was made to dissociate the contributions of these structures by determining the following: 1) careful behavioural descriptions of these rats during post-operative learning of a spatial localization problem, 2) Does similar damage affect spatial localization if the problem has been pre-operatively mastered? 3) Are the impairments specific to the use of one localization strategy, for example a mapping strategy, as opposed to taxis (landmark) or praxis (movement sequence) localization strategies?

Spatial localization learning and retention were assessed using variants of the Morris water task in which rats must escape from opaque water by swimming to a small platform, hidden just beneath the water surface. In different problems, the platform was located at a fixed position relative to the room, at a fixed location relative to the rat's starting locations, randomly positioned, or randomly positioned, but signalled by a conspicuous visual cue. On each trial we recorded the latency to find the platform, the swim distance, the initial swim heading, and the actual swim path. On specific probe trials, we also measured the distribution of swim distance in the four quadrants of the pool.

We reliably dissociated the effects of the different lesions on spatial orientation. In particular, hippocampal damage specifically prevents the use of a mapping strategy, even with extensive pre-operative training. Very similar effects are obtained with enucleation (indicating that distal visual cues are probably necessary for normal, sighted rats to use the mapping strategy). In contrast, frontal cortex damage impairs the learning of a mapping strategy, but not the use of the same strategy after pre-operative training. Electrolytic VMT lesions have a similar effect. Hemidecortication and 6-OHDA lesions slow performance and retard learning in all spatial problems, but do not prevent the utilization of the mapping strategy.

85.17 BEHAVIORAL AND ANATOMICAL EFFECTS OF ADULT VS NEONATAL CEREBRAL HEMISPHERECTOMY. B. Kolb, I. Q. Whishaw and R. J. Sutherland. Dept. of Psychology, Univ. of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

In humans, the side of brain injury and the nature of the behavioral task are important for understanding the neural control of behavior. The different contributions of the two hemispheres can also be modified by early brain damage or by the environmental conditions. Can these variables be studied in a rodent analogue?

We studied the performance of left or right hemispherectomized rats in a battery of tasks in order to answer the following questions: 1) Are the effects of hemispherectomy upon cognitive, complex motor and species typical behavior comparable following adult and neonatal lesions? 2) Is there any asymmetry in the effects of left or right hemispherectomy in infancy or adulthood? 3) Are there anatomical differences in the contralateral hemisphere of adult vs neonatal operates?

Rats with unilateral aspiration lesions of all of the cerebral cortex at 5 or 100 days of age were studied as adults on two spatial tasks (Morris water task, radial arm maze), four motor tasks (puzzle latches, beam traversing, grooming, swimming), several measures of placing and reflexive movements, and several measures of affective behavior. The brains were analyzed for wet weight, cortical thickness, cortical volume, and subcortical dimensions.

The results showed: 1) Hemispherectomy produces a severe impairment in spatial orientation in the water task but not in the radial arm maze, striking abnormalities in placing and reflexive movements but not in more complex motor learning tasks, and abnormalities in affective behavior. 2) Neonatal hemispherectomy produces significant behavioral impairments but there are both qualitative and quantitative differences in behavior compared to adult operates. 3) There is no compelling evidence for differential specialization of the two hemispheres. 4) Neonatal hemispherectomy produces an increase in cortical thickness on the contralateral side as well as several subcortical abnormalities.

These data provide an animal analogue of hemispherectomy in humans and provide a model for the study of anatomical and behavioral recovery processes following brain damage in humans.

85.18 COOLING ANTERIOR TEMPORAL CORTEX SUPPRESSES SHORT-TERM MEMORY AND ENHANCES THE EFFECTS OF INTERFERENCE. James A. Horel, Mary Lou Voytko and Kent G. Salsbury*. Dept. of Anatomy, Upstate Medical Center, Syracuse, NY 13210.

Three monkeys were trained on an automated delayed-match-to-sample (DMS) using randomly presented delays of 0, 15, 30, and 45 sec. and 400 colored slides of objects as stimuli and a liquid reinforcement.

Loops of stainless-steel tubing were implanted over the anterior 9 mm of the temporal lobe. One pair was bilaterally placed over the temporal tip (anterior pair) and the other pair over the anterior extreme of the inferotemporal gyri. These loops could each be separately cooled to suppress the function of the underlying cortex. During experimental trials the temperature of the loop was set at 0° C. Blocks of 20 experimental trials with the loops at this temperature alternated with blocks of 20 control trials with the loops at body temperature.

Cooling either the anterior or posterior pair produced a significant drop in performance at all delays which increased at the longer delays. There was no significant difference between cooling the anterior and posterior pairs. The animals performed between 70-80% correct at the 15 sec. delay. They were then run at this delay with an irrelevant stimulus projected to the center screen for the middle 5 sec. of the delay. Cooling produced a significant increase in sensitivity to this interference which was significantly greater for the anterior than the posterior pair. When the interfering stimuli were presented during the intertrial interval, the effect was weaker.

We then returned to the DMS with the randomly presented delays and suppressed the function of the entire 9 mm area by cooling all four probes. This produced near chance behavior at all delays, including the 0 delay and also produced poor performance on a simultaneous match.

A subsequent study (Voytko et al., 1982 Neuroscience Abstracts) shows that the animals can retain but not learn a visual discrimination with this area cooled. Thus cooling the entire 9 mm suppresses short-term memory but cooling the anterior half of this area increases sensitivity to interference.

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85.19 LEARNING BUT NOT RETENTION AFFECTED BY COOLING ANTERIOR TEMPORAL LOBE. Mary Lou Voytko, Kent G. Salsbury* and James A. Horel. Dept. of Anatomy, Upstate Medical Center, Syracuse, NY 13210.

In a previous study we investigated the ability of three monkeys to perform a visual delayed-match-to-sample (DMS) while the cortex of the anterior 9 mm of the temporal lobe was suppressed by cooling (Horel et al., Neuroscience Abstracts, 1982). When this entire area was cooled, the animals performed near chance at all delays, even the zero delay, or with a simultaneous match. In this study we explored their ability to learn or remember a visual discrimination while this same area was cooled.

The subjects faced 3 rear projection screens. A white light, projected to the center screen started a trial, and a response to it extinguished the light and exposed the discriminative stimuli on the two side screens. The first pair of stimuli were black and white horizontal and vertical stripes, and a response to the horizontal stripes was rewarded with a liquid reinforcement. The correct stimuli alternated sides randomly. They learned the task in one day of 160 trials and showed excellent retention of the task the next day. On the third test day, the anterior 9 mm of the temporal lobe was cooled. The temperature of the cooling probes was 0° C. The animals all showed some degree of initial loss, but they all quickly regained a high level of performance.

The same procedure was used with photographs of objects as stimuli and with the cold applied during acquisition. With the cold on, the animals remained at or near chance for 160 trials. The next day they easily acquired the task without the cold and showed excellent retention of the task on the third test day. On the fourth test day, the cold was again applied, producing a drop in performance, but again the animals quickly reattained a high level of accuracy. This same procedure was repeated with a new pair of photographs. Thus, the animals were unable to learn visual discriminations with the cold applied to the temporal tip, but were able to perform them at a high level if they had learned them before the cold was applied. (Supported by NIH NS18291 and NSF BNS80-40301.)

85.20 THE PULVINAR AND VISUAL INFORMATION PROCESSING IN THE MONKEY, H. V. Soper. Depts. of Anatomy and Psychology, University of Illinois at Chicago, Chicago, Illinois 60680.

The anatomical interconnections between the lateral and inferior divisions of the pulvinar and the visual association cortices have been well established, but it has proven difficult to determine the role these thalamic areas play in processing visual information. Most pulvinar lesion studies show little or no deficits on tasks on which there are substantial deficits following lesions of the prestriate or inferotemporal cortices. This study took a look at the role of the visual pulvinar (lateral and inferior subdivisions) in processing visual information.

Four experimentally naive female cynomologous monkeys (Macaca fascicularis) served as subjects. The testing was conducted in a modified Wisconsin general test apparatus. The animals were preoperatively trained on three tasks and two animals were tested for preoperative retention on each task (not the same animals on each task). For the easy discrimination task the two stimuli were back illuminated. One was a black annulus with orange inside and blue outside. For the other the color locations were reversed. The difficult discrimination was between a white card with a black "plus" on it versus a similar card with the "plus" rotated 45° (an "X"). The criterion for both tasks was 90% correct over three days (90 trials). For the shift task the animal was required to attend to the color (red, blue, or yellow) or form (triangle, pentagon, or octagon) to a criterion of 90% correct within a session (30 trials) before shifting relevance to the other dimension. Criterion for the task was three shifts in six sessions.

Preoperative testing found no 10-day retention losses. Electrolytic lesions were placed bilaterally in the pulvinar, resulting in substantial damage to the inferior pulvinar, some damage to the lateral pulvinar, and very little damage elsewhere. After a 10-day recovery interval the animals had obvious eye movement abnormalities, moderate to severe deficits on the shift task, and minor or no deficits on the easy discrimination task. Three animals had moderate to severe deficits on the difficult discrimination task, but one had only a minor deficit. All these results are similar to ones previously reported for animals with inferotemporal cortex lesions.

The results suggest that the integrity of the visual pulvinar, especially the inferior pulvinar, is essential for normal visual information processing. Curiously, this pulvinar area is anatomically associated with the prestriate cortex.

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86.1 OLFACTORY BULB EEG SPATIAL PATTERNS DURING APPETITIVE CONDITIONING IN RABBITS. G. Viana Di Prisco, W. J. Freeman and G. W. Davis. Department of Physiology-Anatomy, University of California, Berkeley, CA 94720

Spatial patterns of rhythmic high frequency wave activity (bursts) were explored through electrode arrays (8x8) implanted over the lateral surface of the olfactory bulb. Rabbits were trained to give a jaw movement (JM) response when an odor pulse was presented followed by 1 cc of water delivered intraorrally (ISI=2 sec). During conditioning 10 odor trials were given on each session interspersed with blank trials (ITI=60-120 sec). Basal respiratory rate (RR) and spontaneous JMs were assessed during blank trials and familiarization sessions. An on-line computer program was used for automated sniffing (RR) and JM detection and neuroelectric data acquisition (Davis, Freeman and Whitney, Neurosc. Abs., 7:750, 1981). All subjects acquired a RR CR (>50%) indicating that conditioned sniffing behavior can be used as an index for detection of significant odors in appetitive conditioning.

Up to 6 bursts were sampled in each trial before and after CS presentation. Each burst was characterized as the product of its rms amplitude by the cross-correlation coefficient with respect to the ensemble average. The difference between bursts was expressed as $\log_{10}\chi^2$ values (Freeman and Schneider, Psychophysiol., 19:44, 1982). The distribution of these values was computed for blank and odor trials over sessions for all animals. The results show a significant increase in variability within odor trials for pre and post CS burst comparisons especially during the first training sessions. A tendency for burst suppression and changes in low frequency wave activity were observed as well in relation with odor detection.

It is concluded that spatial patterns of burst activity exhibit a quantifiable degree of variability in a motivated state and that increase in fluctuations with conditioning reflects underlying spatially localized changes in synaptic interactions between bulbar neurons(Freeman, Biol. Cyber., 35:221, 1979).

86.2 A PRIORI AND A POSTERIORI TUNING OF A SPATIAL FILTER FOR ODOR-INDUCED INPUT TO THE OLFACTORY BULB OF RABBITS SUBJECTED TO CLASSICAL DELAYED CONDITIONING. W. J. Freeman, G. W. Davis and S. Bressler. Department of Physiology-Anatomy, University of California, Berkeley, CA 94720

Spatiotemporal electroencephalographic (EEG) wave patterns that manifest neural activity in the olfactory bulb and paleocortex are measured in behaving rabbits permanently implanted with electrode arrays (64). The EEG patterns are then simulated with a neural model on a computer by solving sets of 512 1st order nonlinear differential equations. The model and its supporting theory are based on detailed understanding of the anatomical, physiological, psychophysical and pharmacological properties of the bulb and cortex. The data and model show that the EEG spatial patterns depend on memory of prior behavioral experience and not on sensory stimulation alone. This evidence indicates that when an animal undergoes conditioning to a stimulus (CS), the bulb forms a neural template of synaptic connections for that CS, and that the EEG spatial pattern is a manifestation of a search image for that CS. The template can be viewed as a dynamic spatiotemporal filter for an expected CS. When it arrives this filter undergoes an increase in spatial and temporal tuning, indicating that the bulbar output to olfactory cortex is enhanced selectively.

The tuned filter is formed a priori over 1 to 3 sessions of training to respond to an odor and is subject to update by new learning. It is manifested by subtle changes in the spatial pattern of EEG amplitude involving on the order of 1% of bulbar surface area. The change in EEG activity a posteriori with arrival of a CS+ consists of stabilization of a spatial pattern and over-all reduction in amplitude but with relative selective enhancement of certain areas and with an increase in the high end of the spatial power spectrum. There is also an increase in coherence with a shift in temporal peak frequency downward into the pass band of the olfactory cortex viewed as a passive tuned filter. Supported by NIMH 06686.

86.3 RESPONSE OF OLFACTORY BULB AND CORTEX TO CONDITIONED ODOR STIMULATION. S. L. Bressler. Dept. of Physiol.-Anat., Univ. of Calif., Berkeley, CA 94720

This study dealt with the effect on neural activity in the olfactory bulb (OB) and olfactory cortex (OC) of stimulation with odors which an animal has been trained to expect. Neural activity in OB and OC is manifested as 35-85 c/sec sinusoidal bursts in the EEG. Rabbits with chronically implanted electrodes in OB and OC were trained to conditioned stimulus (CS+) odors by following odor delivery with electric shock to the cheek. Other odors (CS-) which were not paired with shock were used as controls. Multichannel EEGs were record-d from an ensemble of 9 depth electrodes in OB. Simultaneous recordings were made from an ensemble of 50 surface electrodes on the OC. Selected for analysis were the one burst (P1) just prior to odor arrival at the mucosa, and the four bursts immediately post-arrival (A1-A4), each burst recorded from the electrode ensembles in OB and in OC. For each of the bursts from P1 to A4, the bulbar and cortical ensemble average burst- were formed. This procedure was followed for 10 CS+ and 10 CS- trials in each of nine recording sessions for each of 4 rabbits.

Analysis of variance was carried out on several measures based on the data base consisting of the OB and OC ensemble averages from P1 to A4 in all sessions of each rabbit. Analysis showed that the amplitude of the bulbar ensemble average significantly declined following arrival of the CS+. The amplitude of the cortical ensemble average however rose significantly from P1 to A1, declining thereafter, but not falling below the P1 level. The ratio of cortical to bulbar amplitudes rose by 32% from P1 to A4. There was also a shift downward in bulbo-cortical co-frequency following CS+ arrival. Co-frequency and amplitude ratio covaried (correlation coefficient = -0.73). Further analysis revealed that the portion of the OB which declined in amplitude to the greatest degree following CS+ stimulation was that part having the greatest phase lag with respect to the bulbar ensemble average. These effects were significant in sessions in which conditioning was established, but not for CS+ trials at the beginning of conditioning or for CS- trials at any time.

Lesion studies have established that the OC depends on OB input to manifest a sinusoidal signal. The present results indicate that OC still manifests a signal in conjunction with overall bulbar suppression. There is further indication that bulbar suppression is spatially selective. This suggests a form of signal inhancement whereby OC receives input from that portion of the bulbar spatial pattern containing information relevant to the CS+. Supported by NIMH 06686.

86.4 ACCESSORY ABDUCENS SINGLE UNIT ACTIVITY DURING NM CONDITIONING IN THE RABBIT. K.J. Quinn, J.F. Disterhoft and C. Weiss. Dept. of Cell Biology and Anatomy, Northwestern University Medical School, Chicago, IL 60611.

Nictitating membrane (NM) extension is a passive consequence of eyeball retraction in the rabbit. Retrograde tracing studies after HRP injections into rabbit retractor bulbi muscles suggest that accessory and principal abducens nuclei contain retractor bulbi motoneurons (Disterhoft and Shipley, 1980, Gray et al., 1981). We have sought to functionally characterize the role of accessory abducens in NM extension by recording from well-isolated accessory abducens neurons in awake, chronically prepared rabbits before, during, and following acquisition of a NM conditioned response. Prior to the recording sessions, a chronic stimulation electrode was implanted on the abducens nerve as it exits the brain stem. Stimulation of the abducens nerve resulted in evoked antidromic field potentials in two regions centered around the principal and accessory abducens nuclei. We report here the activity of neurons lying within the evoked field potential region of the accessory abducens nucleus.

Neurons in accessory abducens have essentially zero spontaneous discharge rate. Single unit activity was observed only during spontaneous NM sweeps or the elicitation of a conditioned or unconditioned NM sweep. Single and multiple unit activity recorded from every electrode track through this region also showed strong trigeminal driving elicited by orbital or periorbital somatosensory stimulation. Unit responses during NM sweep could be divided into three major types. All cell types showed a clear increase in firing highly correlated with onset of the behavioral response. Following this "on" response, cells either: (1) showed a marked decline in rate back to baseline while the NM continued to sweep across the eye ("burst" cells); (2) showed an ongoing, high rate of firing for the duration of the behavioral response ("tonic" cells); (3) showed a partial decline in firing rate followed by a sustained pattern of activity for the duration of the behavioral response ("burst-tonic" cells). Cell firing preceded NM sweep by up to 31 ms. A few units began firing shortly after onset of NM sweep.

Single unit activity in accessory abducens recorded during acquisition of NM CRs showed a high trial-to-trial correlation between the initial appearance of unit firing preceding US onset and the initial appearance of CRs. The trial on which CR unit activity first appeared corresponded to the trial on which behavioral CRs were first detected. We are continuing these experiments to define more precisely the relation of the cellular activity in this region to NM extension.

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86.5 Neuronal Responses of the Rabbit Brainstem and Cerebellum During Performance of the Classically Conditioned Nictitating Membrane/Eyelid response. David A. McCormick*, David G. Lavond*, Nelson H. Donegan* and Richard F. Thompson. Dept. Psychology, Stanford Univ., Stanford, Ca. 94305

Although it is known that cortical/hippocampal regions of the rabbit brain appear to be involved in the classical conditioning of the nictitating membrane (NM)/eyelid response, it is also known that rabbits with all brain tissue removed above the level of the thalamus can learn the standard short delay response. Therefore, by implication, some primary circuit at or below the level of the thalamus must have the ability to encode this learned response. Recent investigations in our laboratory have shown that lesions of the ipsilateral cerebellum, superior cerebellar peduncle, or dentate/interpositus nuclei and surrounding fibers, can abolish the learned response without effecting the unconditioned response or the ability of the rabbit to learn the NM/eyelid response on the contralateral side.

In the present study multiple and single unit recordings were taken from throughout the ipsilateral brainstem and cerebellum of well trained rabbits with a chronic microdrive system. The recording and training trials consisted of a 350 msec. acoustic CS followed after 250 msec. by a 100 msec. corneal airpuff UCS. Histograms of the neuronal recordings were computer generated and analyzed in relation to the onset of the CS, the UCS, or the onset of the conditioned response.

Structures with onset latencies close to that of the conditioned response were found in the following sets: A.) Those structures which directly control the conditioned movements (5^{th}, 6^{th}, accessory 6^{th}, and 7^{th} nuclei), B.) Those structures which relate to the somatosensory feedback of the movement (sensory 5^{th}, spinal 5^{th}), C.) Restricted regions of the cerebellum and its related brainstem nuclei (pontine nuclei, tegmental nucleus of the pons, red nucleus, inferior olive), D.) superior colliculus and periaquaductal gray, and E.) various reticular regions. The presence of reponses within structures known to be contralateral may be explained by the fact that in most animals the conditioned eyeblink response is to some degree bilateral. Responses relating to the onset or the offset of the tone and airpuff were found in all brainstem auditory nuclei, the periaquaductal gray, the superior colliculus, and various reticular regions, as well as within restricted regions of the cerebellum. No responses relating to the onset of the conditioned responses were found within the classical auditory structures.

These data, with the aforementioned lesion data, imply that the cerebellum and its related nuclei in the brainstem are a major and critical part of the primary brainstem circuit encoding the learned NM extension/eyeblink response.

86.6 DISCRIMINATIVE CONDITIONING OF EYEBLINK WITH AVERSIVE BRAIN STIMULATION. N.E. Berthier, B. Betts* and C.D. Woody. Depts. of Anatomy and Psychiatry, UCLA Med. Center, Los Angeles, CA 90024.

Classical conditioning was studied in a mammalian preparation in which a discriminative response with multiple latency components could be rapidly acquired (Berthier et al., Abstr. Soc. Neurosci., 7:750, 1981). During training, the CS (75 db click) was followed 340 ms later by mechanical tap of the glabella (US) which elicited a bilateral eye blink (UR). Electrical stimulation of the hypothalamus (HS) was delivered unilaterally 580 ms after the CS. A discriminative stimulus (DS, 75 db hiss) was delivered 4.4 s after the CS. The motivational properties of the HS were tested in both passive and active avoidance paradigms using a step down procedure. During passive avoidance training, stepping off the platform was followed by 2 trains of HS and removal from the apparatus. In the passive avoidance paradigm, the latency to step down increased to five minutes or more by the fifth trial. When the procedure was reversed, with cats receiving a train of HS every 10 sec while on the platform, average step down latency decreased to less than 10 sec by the 8th trial. These results indicate the HS could be used as a negative reinforcer and presumably had aversive properties. Voronin et al. (Br. Res., 92:385-402, 1975) used a positive HS to produce conditioning. The successful use of a negative HS suggests that the "motivational" aspect may not be crucial for the rapid development of the CR.

Discrimination training with this HS led to discriminative responding in most cats. Cats initially responded to both the CS and DS but by the 15th trial animals showed discriminative responding to the CS. Previous reports showed that this responding is associative (Kim and Woody, Abstr. Soc. Neurosci., 5:319, 1979). Reversal of the click and hiss (i.e., hiss=CS, click=DS) led to a rapid decrease in discriminative responding to the click followed by an extended period where the animals were responding equally to the CS and DS. Reversal responding was accomplished with difficulty, and could not be obtained in all cats. Reversal conditioning again demonstrates the associative nature of this form of conditioning. The difficulty in suppressing responses learned originally to the click as CS, despite the hiss having equal intensity and greater overall energy than the click, demonstrates the long lasting retention of learning of this type.

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86.7 HIPPOCAMPAL ACTIVITY DURING APPETITIVE CLASSICAL CONDITIONING IN RABBITS. S.D. Berry and C.G. Oliver*. Dept. of Psychology, Miami Univ., Oxford, OH 45056.

Chronic recordings of multiple unit activity (MUA) and EEG from the dorsal hippocampus were made during appetitive jaw movement conditioning in 10 New Zealand White rabbits. Subjects were implanted with stainless steel electrodes (5-7 u tip, 30-40 u exposed shaft) in the CA1 pyramidal cell layer. After 1 week recovery, animals were placed on a 23 hr water deprivation schedule and adapted to the restraint/conditioning apparatus. Two daily sessions of 54 trials were conducted using a tone conditioned stimulus (CS, 1 KHz, 85 dB, 350 msec) and a saccharin unconditioned stimulus (UCS, .22%, 1 cc, 100 msec duration). The CS-UCS interval was 250 msec and the intertrial interval averaged 60 sec. Every 9th trial was a tone-alone test trial. Control subjects were given explicitly unpaired tone and saccharin presentations. Analysis consisted of poststimulus histograms of integrated MUA and filtered (2-25 Hz) EEG activity.

Behaviorally, the conditioned response (CR) trained very rapidly (all subjects emitted CRs within 20 trials) and, over training, began to precede UCS occurrence. Control animals showed few CRs to tone presentation and long-latency responses to the UCS.

EEG responses consisted of theta or rhythmic slow activity (RSA) of approximately 8 Hz, triggered by CS onset. Conditioned unit activity displayed a clear increase to the tone CS, was typically rhythmic (7-9 Hz), and occurred at a much shorter latency (<100 msec) than the behavioral CR (>200 msec). Both tone- and saccharin-evoked responses were observed in control animals.

These results differ in some respects from hippocampal activity observed during aversive nictitating membrane conditioning in the rabbit, suggesting that hippocampal activity may respond to motivational conditions during learning.

86.8 AUGMENTATION OF DENTATE GRANULE CELL POPULATION SPIKES AFTER HIGH FREQUENCY TRAINS DELIVERED TO THE SEPTUM AND THE PERFORANT PATH. Bryan D. Fantie Dept. of Psychology, Dalhousie University, Nova Scotia, Canada B3H 4J1

This preliminary investigation examined the effect of simultaneous high frequency stimulation (HFS) delivered to both the perforant path (PP) and the medial septum (MS) on the field potential subsequently evoked by a test stimulus (applied to the PP) or a pair of test stimuli (applied to both PP and MS) and recorded from the fascia dentata of six pentobarbital anaesthetized rats.

Stimulating the entorhinal cortex produces a large extracellular field potential in the ipsilateral hippocampus which is generally interpreted as an average excitatory postsynaptic potential (EPSP). If stimulation is sufficient, a negative-going population spike (PS) appears superimposed upon the positive EPSP waveform and is interpreted as representing the almost synchronous discharge of many granule cells (Lømo, Exp. Br. Res.,12:18,1971). Pairing single pulse stimulation of the PP and MS augments the PS (Fantie & Goddard, Br. Res., in press). HFS of the PP alone causes long periods of marked increase in response to a test stimulus (Long Term Potentiation, LTP)(Douglas & Goddard, Br. Res.,86:205, 1975).

Overlapping HFS trains, increasing in intensity over the series and sufficient to reliably produce LTP, were delivered every 20 minutes to the MS and one PP. The contalateral PP received similar HFS but it was arranged so that the trains delivered to each hemisphere were separated by 10 minutes as the MS sends projections to both hippocampi. In this way, the two hemispheres could be compared as the only difference in their treatment was the temporal sequencing of the stimulus trains.

Following the conditioning series, the EPSP portion of the evoked field potential recorded from the side which had received paired HFS was found to have been "potentiated" more than the side which had received unpaired HFS. There was a great deal of variance between subjects. When PP and MS pulses were paired, the septal modulation of the PS without alteration of the EPSP was dramatically enhanced only in the hemisphere in which the MS and PP HFS had been synchronous.

Since the medial septum exerts its influence bilaterally, both hippocampi had received comparable input from their afferents. As this enhanced augmentation depended upon the temporal contiguity of the MS and PP conditioning trains, an associational mechanism appears to be involved and may provide a neuronal model for "physiological context".

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86.9 EFFECTS OF POST-TETANIC POTENTIATION ON SUBSEQUENT GENERATION OF ENHANCEMENT. P. E. Sharp and B. L. McNaughton. Dept. of Psychology, Univ. of Colorado, Boulder, Colorado 80309.

The increase in synaptic efficacy following tetanic stimulation of the perforant path-fascia dentata system consists of several superimposed processes (McNaughton, J. Physiol. 324, 1982). Two short-term processes, augmentation and potentiation (collectively known as PTP) involve increases in transmitter release. The control mechanism of the long-term process, known as LTP, or enhancement, has not yet been determined. This study examined whether increased transmitter release due to PTP would increase the effectiveness of a subsequent enhancement generating treatment given to the same pathway. PTP can be produced independently of enhancement, since the former requires stimulation of only a few fibers, while enhancement requires simultaneous stimulation of a large number (McNaughton et al., B.Res. 157, 1978). Since Dunwiddie et al. (B.Res. 150, 1978), have shown that reduced transmitter release caused by lowering [Ca^{++}] prevents enhancement, we predicted that increased transmitter release would increase enhancement. In one condition an enhancing treatment (tetanic stimulation of both the lateral and medial pathways simultaneously) was administered just 5 sec after a similar burst of stimulation to the lateral pathway only (which produced only PTP, elevating the response by 104% at this interval). In a second condition, there was a 155 sec interval between the two treatments, so that PTP had decayed to near zero before the enhancing stimulus. Responses to single impulses to the lateral pathway were tested throughout the experiment. Both conditions were presented to each of 16 rats; the two hemispheres serving as matched samples. While the overall mean enhancement at 20 min was 45%, there was no significant difference between the amounts of enhancement with or without prior generation of PTP. In fact, the 15% difference observed was opposite to that predicted. One possible explanation of this result is that some presynaptically released factor (other than the transmitter) which is not subject to PTP, regulates the generation of enhancement. It remains to be demonstrated, however, that the total amount of transmitter release over the course of the entire enhancement conditioning train was actually different between the two groups.

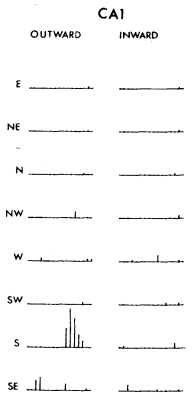
It has been suggested that long-term enhancement could be a substrate for associative memory. The requirement for simultaneous stimulation of many afferents fits in well with this notion. It provides the possibility that more than one input must be present if association between stimuli is to occur. The current results indicate that the immediately preceding activity of one set of afferents would not increase their ability to enter into subsequent associations.

86.10 DIRECTIONAL SPECIFICITY OF RAT HIPPOCAMPAL "PLACE CELLS" ON A RADIAL 8-ARM MAZE, B. L. McNaughton, C. A. Barnes, J. O'Keefe*. Dept. Anat. & Embryol., University College, London WC1E 6BT, U.K.

Single unit activity was recorded from the hippocampus of rats on an 8-arm maze. Animals were trained to obtain food reward at the end of the arms. Entry to the arms was controlled, so that all 8 arms were visited once in a random sequence on a given trial. For each cell, 8 such trials were given to assess firing consistency. The position of a light-emitting diode on the animal's head, and the time of occurrence of each spike was monitored by digital computer.

For analysis, each arm was divided into 16 bins for each of the outward and inward radial directions. The total number of spikes per bin was divided by the occupancy time of that bin to give the average firing rate. Two principal methods of quantitation were used. Specificity was defined as the rate on the preferred arm in the preferred direction divided by the mean rates on all the other arm-directions. For the directionality index, the rate ratio was taken in the preferred to non-preferred directions for the arm with the highest rate.

Complex spike cells were recorded from CA1 (n=27), CA3 (n=15), and fascia dentata (n=5). In agreement with previous reports, place specificity was found in all areas studied. For example, the specificity index for CA1 was 8.2 $\pm$ 3 (S.E.M.). In addition however, most cells showed marked directional preference as well. The average directionality index for the CA1 cells was 8.9 $\pm$ 3 (S.E.M.). Thus, on average, the firing rate in the non-preferred direction, on the preferred arm, was not significantly different from the rate on any other arm. In CA1 there was no overall preference across cells for any particular arm or direction. CA3 cells, however, had a marked preference for inward, frequently firing at high rates in this direction on several or all arms. The specificity and directionality of a CA1 cell is illustrated. Each historgram represents the distribution of firing rates for a given arm-direction averaged across 8 trials. The center of the maze is at the left of each histogram.



In conclusion, both orientation and location are important determinants of hippocampal complex spike cell activity in the rat, at least within the geometrically restricted environment studied here.

86.11 LONG-TERM POTENTIATION IS IMPAIRED BY ADRENALECTOMY AND RESTORED BY CORTICOSTERONE. R. C. Dana*, R. A. Gerren, D. B. Sternberg, J. L. Martinez, Jr., J. Hall*, N. A. Stansbury*, and N. M. Weinberger. Dept. Psychobio., Univ. of Calif., Irvine, Irvine, Calif. 92717.

Hormones of the pituitary-adrenal axis have widespread modulatory effects upon behavior and brain physiology, e.g. learning, memory, and synaptic excitability. The hippocampus is implicated in learning and memory and exhibits synaptic plasticity as evidenced by a phenomenon known as Long-Term Potentiation (LTP). As the hippocampus contains neurons with large numbers of corticosteroid receptors, and since these same neurons may be involved in LTP, we investigated the effects of altered hormone levels upon the development of LTP. Adrenalectomized (ADX) rats were compared to normal and sham-ADX controls in their ability to develop LTP before and after corticosterone (B) administration.

Male Sprague-Dawley rats were anesthetized with Nembutal and bipolar stimulating electrodes were placed in the perforant path. Test stimuli consisted of 300-400μA, 0.1 ms pulses delivered at 0.2 Hz. Characteristic dentate gyrus monosynaptic responses were recorded. Parameters of this response studied included: 1) slope of the EPSP, 2) amplitude of the population spike, and 3) latency of the population spike peak. After a stable baseline response was established (10-30 min), four brief (250 ms duration) high-frequency trains (HFT) were presented to the perforant path at 200 Hz, with one second inter-train intervals. Test stimuli were recorded for at least 40 min post-HFT.

Normal rats demonstrated 50-600% potentiation of the amplitude of the population spike, significant increases in the slope of the EPSP, and significant decreases in the latency of the population spike, which lasted for the duration of the recording session. The amount of LTP appears to be correlated with the level of plasma B; the greatest percentage increase coinciding with peak levels of B. This finding suggests that the diurnal variation of B may modulate LTP.

ADX rats failed to develop significant LTP during the 40 min post-HFT period. When ADX rats received 300μg of B (I.P.) and identical conditioning trains presented 30 min later, the ability to develop LTP was restored. At least a 100% increase in the amplitude of the population spike was observed. The sham-ADX control group did not differ significantly from the normal group.

These findings indicate that corticosteroids may modulate synaptic plasticity, and further suggest that hormones of the pituitary-adrenal axis may regulate complex brain processes. (Supported by NIAAA grant AA03506-05 to N.M.W. and ONR contract N00014-82-K-0385 to J.L.M) We acknowledge advice from J. Gaddy and G. Barrionuevo in performing these experiments.

86.12 MODULATION OF LONG-TERM POTENTIATION (LTP) BY ADRENERGIC AGONISTS. R.L. Delanoy, J.S. Merrin and P.E. Gold. Dept. of Psychology, University of Virginia, Charlottesville, VA. 22901.

Long-term potentiation is characterized by a long-lasting alteration in a monosynaptic evoked response following high-frequency stimulation. Because LTP has rapid onset and long duration, it may reflect a form of plasticity used in memory storage. The present experiment examines the possibility that memory modulating adrenergic agents can also modulate LTP.

In rats under pentobarbital anesthesia, monopolar recording and bipolar stimulating electrodes were implanted in the hilus of the dentate gyrus and the angular bundle, respectively. Test stimuli (100 usec duration) were delivered at a rate of one pulse every 30 sec throughout the experiment. After an initial 10 min stabilization period, the test stimulus intensity was adjusted so that the population spike approximately equalled the EPSP amplitude. Upon establishing this baseline evoked response, animals received an IP injection of saline, d-amphetamine (.001, .01*, .1*, 1.0*, 3.0*, 10.0, and 15.0 mg/kg), epinephrine (.001, .1*, 1.0 mg/kg) or clonidine (.01, .1, 1.0*, 10.0 mg/kg). At 15-20 min after injection, minor adjustments in test stimulus intensities were again made, if necessary, to reestablish the baseline evoked response. Approximately 5 min later, 10 high-frequency trains (200 Hz, 100 usec pulse duration, 0.4 sec train duration, every 10 sec) or sham stimulation (stimulator off) were delivered. Population spike amplitudes were measured at 15-20 min after high-frequency stimulation and were expressed as percent change from pre-stimulation amplitudes.

Each of the drugs tested thus far (amphetamine, epinephrine, and clonidine) significantly augmented LTP. In each case, significant facilitation was observed with intermediate doses (* above; approximately two-fold increases) but not at higher or lower doses. None of the drugs significantly altered the unpotentiated population spike amplitude.

These results indicate that the mechanisms which mediate LTP can be modulated by adrenergic stimulation. Because epinephrine does not cross the blood-brain barrier, LTP modulation may result in part from the stimulation of peripheral adrenergic receptors. Previous studies of memory modulation with amphetamine, epinephrine and clonidine demonstrate dose response relationships very similar to those observed with LTP modulation. The similarities between drug modulation of LTP and memory support the use of LTP as an analog of memory.

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86.13 NALOXONE ANTAGONISM OF GRANULE CELL STIMUALTION-INDUCED MEMORY DISRUPTION: NOT AN ANTI-EPILEPTIC MECHANISM. Timothy J. Collier and Aryeh Routtenberg. Cresap Lab., Northwestern Univ. Evanston, IL. 60201.

We have previously shown that electrical stimulation of rat dentate gyrus granule cells (GC) disrupts memory performance in a radial maze task (Collier et al., Behav. & Neural Biol., in press). Consistent with histochemical evidence of enkephalin in these cells (Gall et al., JCN, 198:335, 1981), memory disruption produced by GC stimulation can be prevented by pretreatment with the opiate antagonist naloxone (Collier et al., Neurosci. Abst. 7: 359, 1981). If GC stimulation is producing seizure activity in a memory-promoting system, then one mechanism by which naloxone may protect memory is by prevention of these afterdischarges. To examine this possibility, 3 male albino rats were implanted with chronic electrodes to record the activity of the GC layers of both hemispheres and the CA3 zone ipsilateral to a GC stimulating electrode. Recordings were carried out in the presence and absence of a systemic dose of naloxone (1.0mg/kg). Brain stimulation (60Hz sine waves, 5 μA peak amplitude for 10 sec.) identical to that which is memory disruptive was employed. At this stimulus intensity, 2 of 3 animals exhibited high amplitude synchronous activity on 6 of 16 tests (37.5%). Further tests indicated that a 25μA stimulus was required to elicit an abnormal electrographic response on every test. The third animal slowly developed a seizure-like response, showing no abnormal activity on the first 5 tests, but consistently showing brief episodes of high amplitude spiking over the last 6 tests. Naloxone pretreatment had no effect on any phase of stimulation-induced abnormal electrographic activity (5 tests). Thus, the potency of our stimulation for producing working memory impairment (disruptive on 26 of 38 tests-68%) does not appear to be completely explained by seizure generation, nor can naloxone's memory-protective influence be linked to an anti-epileptic mechanism. At least two alternative explanations deserve consideration: 1) GC stimulation may activate an opioid peptide-mediated working memory erasure system, with naloxone preventing erasure, at least in part, by an action at the GC-CA3 synapse; 2) naloxone may be acting at a site outside the hippocampal formation which is activated by GC stimulation, and can modify memory performance. Supported by MHNS 16097 to T.J.C. and NIMH 25281 to A.R.

86.14 A TECHNIQUE TO DETECT AND CHARACTERIZE NEURONAL RESPONSES DURING LEARNING. S. D. Woods*[1], T. C. Trusk*[2] and E. A. Stein[2]. Depts. of Mathematics[1] and Biology[2], Marquette Univ., Milwaukee, WI 53233

Our laboratory is investigating the behavior of single neuronal responses during the course of operant behavior. The training protocol utilizes up to three different types of reinforcers (i.e., milk, intracranial self-stimulation, morphine sulfate) each paired with one of three different frequency tones. A PDP 11/34 controls all aspects of stimuli presentation and data collection.

We observed that changes in unit activity resulting from operant learning were not readily discernible by standard peristimulus time histograms. Thus, we have developed analyses that allow us to better characterize unit activity throughout the course of learning.

Our analysis is based on the assumption that the PSTH is the superposition of two terms: the neuronal response to 1) the applied stimulus and 2) to all noncontingent sources. To separate these two responses, we systematically employ a series of weighted moving averages in which the weights are chosen to ameloriate the expected stimulus response and minimize the remainder. If the height of the PSTH at time t is X_t then a weighted moving average has the form: $Y_{t'} = \sum_{t=t'}^{t'+N} W_t X_t$ where the W_t's are the weighting coefficients. The resulting variable $Y_{t'}$ represents an enhancement of the original PSTH. The parameter N is the time interval over which the average is computed. This interval or window is adjusted depending on the particular record being considered. These adjustments enhance selected aspects of the original histogram. Examples include: $W_t=1$ (moving average) and $W_t=e(t-t'+N/2)^2$ (gaussian weighted average). In the sense that the weighted moving average is a discrete version of a convolution, the weighting coefficients are the kernel of this linear operation. This kernel is the fourier transform of the transfer function and can be viewed as a filter which decreases the effects of noise.

To determine W_t and N we performed a series of pseudoconditioning experiments whereby neuronal responses were recorded during random presentations of the six stimuli irrespective of the rats' behavior. Resulting spike trains were converted first to PSTHs for each of the 6 stimuli. Comparison of these histograms and their FFTs with those obtained during stimulus-free conditions (i.e., "spontaneous activity"), allowed us to choose weights and windows that enhance the S/N ratio of each neuronal response. The transformed data is then amenable to simple statistics (e.g., t tests) to detect stimulus-bound responses, their latency and duration. Supported by NIDA grant #DA02334.

86.15 PHYSIOLOGICAL PLASTICITY OF SINGLE NEURONS IN SECONDARY AUDITORY CORTEX (AII) DURING PUPILLARY CONDITIONING IN CAT. D.M. Diamond* and N.M. Weinberger. Dept. Psychobiology, Univ. of Calif. Irvine, Irvine, CA 92717.

Previous studies of neuronal discharges in the thalamocortical auditory system during behavioral conditioning with acoustic signals have revealed differential plasticity in the medial geniculate nucleus (MGN); a lack of plasticity in the tonotopically-organized ventral division (MGv) and a high degree of plasticity in the non-tonotopically-organized magnocellular division (MGm). Primary auditory cortex (AI), which receives convergent input from these two divisions of the MGN, has an intermediate degree of plasticity during learning. (Hopkins & Weinberger, Neurosci. Abst. 1980, 6, 424). The present study characterized the activity of single neurons in the secondary auditory cortical field (AII) during pupillary conditioning. AII is innervated by MGm, but not by MGv.

Single unit activity was recorded during acquisition of the pupillary dilation response in chronically-prepared cats under neuromuscular blockade and artificial respiration (Oleson et al., Beh. Biol., 1972, 7, 829). Subjects were trained with tone or white-noise signalled pawshock. A sensitization phase, consisting of non-paired presentation of these stimuli, preceded pairing and controlled for non-associative factors. Data were obtained from one neuron in a single training session. Subjects were routinely involved in more than one training session at intervals of at least one week.

Data were obtained from 22 cells, histologically verified in AII. All twenty-two cells developed a statistically significant increase or decrease in background discharges during stimulus pairing, relative to the sensitization phase; 17/22 developed a decrement, 5/22 had an increment. The rate of decrease in background activity was directly related to the rate of acquisition of the pupillary dilation conditioned response. Changes in evoked activity developed in 21/22 neurons; increases and decreases were approximately equally probable. The direction of evoked changes was not related to the direction of changes in background activity.

These findings reveal that there is a much higher probability of the development of discharge plasticity in AII than AI. The extraordinary degree of plasticity in secondary auditory cortex may be due in part to the fact that it receives convergent input from plastic regions, i.e., MGm and AI, and lacks input from the non-plastic thalamic auditory nucleus, MGv.

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86.16 DISCRIMINATIVE NEURONAL ACTIVITY IN THE RABBIT CINGULATE CORTEX: TRACE AND DELAY PARADIGMS COMPARED. S. Sparenborg*, R. W. Lambert*, R. Maiorca* and M. Gabriel. (SPON: C. Erickson). Dept. Psychol., Univ. Texas, Austin, TX 78712.

Discriminative neuronal activity develops in the cingulate cortex in rabbits during differential avoidance conditioning. That is, a greater neuronal discharge develops to the CS^+ (a tone initiated five seconds in advance of the footshock US) than to the CS^- (a tone not followed by the US). These results, together with the several reports of behavioral impairment due to cingulate cortical damage, suggested that the cingulate cortex is involved in behavior-relevant encoding of the associative significance of the conditional stimuli (see Gabriel et al., Science, 208:1050, 1980). The past studies with this approach have involved delay conditioning, wherein the CS^+ remains on until US onset or until a behavioral response is made. If the discriminative discharges reflect associative encoding of CSs, it might be expected that a brief duration CS would constrain cingulate cortical processing to the interval of stimulus presentation. Such constraint may result in a greater discriminative discharge to the CSs. To test this idea three rabbits with a total of five electrodes in the cingulate cortex were given trace conditioning wherein the CS was presented for 1.1 sec and the US was presented 5.0 sec after CS^+ onset. Three subjects trained with the delay procedure were matched to the trace subjects for number (5) of recording sites, amplitude of the neuronal records, and laminar position of the electrodes. The trace subjects required a mean of 10.3 sessions to reach the behavioral criterion, whereas the delay subjects required 3.3 sessions. Analysis of variance revealed significant discriminative neural activity in the trace conditioning group during the session of the first significant behavioral discrimination and during the session in which the criterion behavioral discrimination was attained. There were no significant discriminative effects in the delay conditioning group. Past studies with delay conditioning yielded significant discriminative effects but these studies involved 10-20 cortical records. Absence of significant discriminative activity in the present delay group is attributable to the small sample. The significant discriminative activity despite the small sample in the trace group supports the idea that as CS duration is reduced, cingulate cortical involvement in discriminative stimulus processing is enhanced. These data are compatible with previous results (Weisz et al., Bull. Psychon. Soc., Abstr. 194, 1980) indicating that limbic cortical structures (i.e. hippocampus) are preferentially involved in mediating behavioral learning with trace as opposed to delay conditioning. (Supported by NIMH grant 31351 to M. G.).

86.17 NEURONAL ACTIVITY OF THE BRAINSTEM RETICULAR FORMATION DURING DISCRIMINATIVE AVOIDANCE LEARNING IN RABBITS. B. Gregg*, M. Kittrell, W. Dailey*, A. Clancy*, and M. Gabriel. Dept. Psychol., Univ. Texas, Austin, TX 78712.

Multiple-unit activity was recorded in the brainstem reticular formation (RF) in rabbits during discriminative conditioning of locomotory (wheel-running) avoidance behavior. The conditional stimuli (CS^+ and CS^-) were pure tones differing in frequency and the unconditional stimulus (US) was a constant-current footshock (1.5-2.5 Ma.) delivered through the grid floor of the wheel. The rabbits received daily sessions of differential conditioning in which the CS^+ (initiated 5 seconds in advance of the US) and the CS^- (never followed by the US) were presented 60 times each, in a random sequence. Locomotion during the CS^+ terminated the CS^+ and prevented footshock. To permit pooling of the data of different rabbits and to facilitate comparison of the present and past data, assessments of the CS evoked neuronal activity were made at certain behaviorally-defined stages of conditioning (the first exposure to training, the session of the first significant discriminative behavior, the session in which behavioral criterion (asymptote) was attained, and the postcriterial "overtraining" sessions).

Recording loci at both pontine ($\underline{N}$ = 11) and mesencephalic ($\underline{N}$ = 6) levels of the RF manifested neuronal discharges to the tonal stimuli. However the pontine but not the mesencephalic loci manifested development of discriminative discharges (i.e. a significantly greater discharge to the CS^+ than to the CS^-) during the course of behavioral acquisition. Moreover, among the pontine loci the more rostral placements ($\underline{N}$ = 4) showed clear discriminative discharges at brief latency (10-20 msec) during the first exposure to the conditioning procedure. The caudal pontine loci ($\underline{N}$ = 7) did not manifest discriminative activity until the session of first significant behavioral discrimination. All pontine loci manifested a diminution of the discriminative discharges during the late (criterial and postcriterial) stages of training. Of the several brain systems examined to date with these procedures, the rostral pontine RF and the medial prefrontal cortex (E. Orona et al., Neurosci. Abstr. 245.6, 1981) are the first to manifest discriminative activity at brief latency, suggesting a role for these structures in the initiation of behavioral learning. (Supported by NIMH grant 26276 to M. G.).

86.18 ANTERIOR THALAMIC LESIONS BLOCK THE DEVELOPMENT OF DISCRIMINATIVE NEURONAL ACTIVITY IN THE CINGULATE CORTEX DURING BEHAVIORAL LEARNING IN RABBITS. M. Gabriel, R. W. Lambert*, S. Sparenborg* and R. Maiorca*. Dept. Psychol., Univ. Texas, Austin, TX 78712.

Discriminative neuronal activity in the form of a greater neuronal discharge to a CS^+ (a tone initiated 5 seconds in advance of a footshock US) than to a CS^- (a tone predicting no US) develops during acquisition of discriminative (locomotory) avoidance behavior in rabbits. The discriminative activity develops in the cingulate cortex in the early stages of training, and it develops in the reciprocally interconnected AV thalamic nucleus in the late stages of training (Gabriel et al., Science, 208:1050, 1980). AV thalamic electrolytic lesions after training attenuated the performance of the avoidance behavior during a post-lesion retention test. Also, the excitatory discriminative discharges in controls were replaced in lesioned subjects by non-discriminative and inhibitory ("off") neuronal responses (Gabriel et al., Soc. Neurosci. Abstr. 245.6, 1981). These results suggested that the integrity of the AV nucleus is essential to the functioning of the cingulate cortex and to the normal performance of the learned behavior. Yet, the late development of the discriminative activity of the AV nucleus, suggested that the cortex may be a "stand alone" processor in the early training stages. Thus destruction of the AV nucleus prior to training may not alter the functioning of the cingulate cortex in these early stages. To test this, AV nuclear lesions were given to 6 rabbits followed by training to criterion and a retention test (extinction, then reacquisition) as in the previous study with post-training lesions. The lesioned subjects were compared throughout training and retention testing, to the subjects given post-training lesions ($\underline{N}$ = 7) and to sham operates ($\underline{N}$ = 5). The lesions did not significantly affect the behavioral acquisition or retention. However, they did block the manifestation of excitatory, discriminative activity in the cingulate cortex in all stages of training and retention. Significant inhibitory "off" neuronal responses to the CSs, developed during the late stage of training and were present during the retention test. These off responses were significantly more pronounced in the ventral (retrosplenial) subarea of the cortex than in the dorsal cingulate cortex. There were no differences at the outset of training, in the magnitude of the off-response in subjects lesioned 1 or 12 days before training, suggesting that the development of the off responses depended on training, not on the passage of time. It is suggested that the inhibitory neuronal responses result from subicular afferents to the cingulate cortex, which increase their influence on cingulate cortical activity after removal of the excitatory afferents of AV nuclear origin. (Supported by NIMH grant 31351 to M. G.).

86.19 EFFECTS OF MIDLINE LIMBIC CORTICAL LESIONS ON DISCRIMINATIVE AVOIDANCE BEHAVIOR AND NEURONAL ACTIVITY IN THE NEOSTRIATUM. R. W. Lambert* and M. Gabriel. (SPON: W. R. Salafia). Dept. Psychol., Univ. Texas, Austin, Tx 78712.

Past studies have demonstrated the development of discriminative multiple-unit activity in the midline limbic (prefrontal and cingulate) cortical areas during differential conditioning of locomotory avoidance behavior in rabbits (Orona, E., Soc. for Neurosci. Abstr. 148.13, 1980; Gabriel, M., et al., Science, 208: 1050, 1980). That is, a greater neuronal discharge developed to the CS+ (a tone initiated 5 seconds in advance of the footshock US) than to the CS- (a tone not predictive of the US). These results, together with several studies indicating that damage to midline cortical areas retards behavioral acquisition, suggest that the discriminative neuronal discharges may be causally significant for the acquisition of the learned behavior. Yet the pathways whereby the neuronal discharge may influence the behavior have not been identified. The extensive projections from midline limbic cortical areas to the neostriatum suggest that this may be an important pathway for this influence. Here we examine the possibility that input from the prefrontal and cingulate cortices may be essential for the normal functioning of neostriatal neurons and for the acquisition of the learned behavior.

Prior to training, five rabbits received bilateral electrolytic lesions of the prefrontal and cingulate cortical areas and chronically indwelling multiple-unit electrodes were implanted in the neostriatum (4 placements in the caudate nucleus; 3 in the putamen). Nine additional rabbits were similarly implanted (10 placements in the caudate nucleus; 2 in the putamen), but did not receive lesions. Behavioral performance of the differential locomotory avoidance response was disrupted significantly (Days to behavioral criterion: Controls $\bar{X}$ = 7.56; Lesioned $\bar{X}$ = 13.60: 3 of 5 lesioned subjects, but no controls failed to achieve criterion after 15 daily conditioning sessions). In contrast to the disruptive effects on behavioral acquisition, an enhanced neuronal discrimination developed in the neostriata of lesioned subjects, relative to controls. This effect was evident in the first conditioning session and it persisted through the final session. Thus lesions blocked behavioral acquisition and they influenced the activity of the neostriatum. However, the influence was of an unexpected variety: neuronal discrimination which was absent in controls, developed in the lesioned subjects. This result suggested that the neostriatum may be involved in compensatory processes following limbic cortical damage. (Supported by NIMH grant 31351 to M.G.)

86.20 LONG LATENCY EVENT RELATED POTENTIALS (ERPs) IN MONKEY. D.J. LEE* and A. STARR. Depts. of Psychobiology and Neurology, California College of Medicine, University of California, Irvine, CA 92717.

The late positive component of the human ERP, P300, is an endogenous component whose amplitude is related to at least three interactive factors: 1) task relevance, 2) stimulus probability, 3) the precise sequence of preceding stimuli. A typical method for eliciting P300 in humans is the "oddball" paradigm, in which subjects are instructed to count target tones occurring in a sequence of more frequent non-target tones. This task requires attention to both tones, while the target tone alone requires special processing (i.e., count) and elicits the P300 component.

Utilizing a variant of the oddball paradigm, we investigated the monkey (Macaca nemestrina) ERP along three dimensions: 1) waveform features (components), 2) relation of components to stimulus and behavioral changes, 3) scalp distribution. Three monkeys were operantly conditioned to discriminate an infrequent target tone (CS+:1 KHz, 400 ms duration) and frequent non-target tone (CS-:2.5 KHz, 400 ms duration). Monkeys were required to press and hold a lever to start the computer controlled, random sequence of tones. The lever needed to be released at CS+ offset for a juice reward, and then immediately re-depressed to continue the stimulus sequence. This procedure promotes attentiveness to both tones, while the target tone (CS+) alone is task relevant.

Following training, monkeys were implanted with 20 screw electrodes placed according to the 10-20 system. The EEG (all electrode locations were referenced to a non-cephalic site, 1.5cm below the inion), EOG and lever responses were recorded on magnetic tape, digitized and averaged off line. The potentials elicited by the rare CS+ were larger in amplitude for four components: N70, P130, N210, and P300.This sequence of components in monkey resembles ERPs recorded from human subjects under similar condititons. Furthermore, the primate P300 decreases in amplitude when the signal probability is increased. The primate P300 requires task relevance. If the lever is removed from the animal's control during the stimulus sequence, the P300 disappears. The primate P300 is of maximal amplitude over the central and parietal scalp, similar to the human P300. These preliminary results suggest the primate may serve as a model of the human P300 component for further anatomical and physiological studies of neural processes giving rise to P300.

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87.1 EFFECT OF 6-HYDROXYDOPAMINE ON CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE RESPONSE. L. Winsky* and J. A. Harvey. (SPON. R. W. Lind). Dept. of Psychology, The University of Iowa, Iowa City, Iowa 52242

Previous investigations of the effects of monoamine depletions on learning have generally employed the use of operant techniques. This study was designed to examine the effects of such depletions on a classically conditioned response through the use of 6-hydroxydopamine (6-HDA). 6-HDA doses of 270, 540, or 670 µg (as the base) were injected into each of the lateral ventricles of the albino rabbit. Control animals received saline-ascorbic vehicle. Following a recovery period of approximately 10 days, rabbits were given 10 daily sessions of classical conditioning. Each session consisted of presentations of tone or light conditioned stimuli (CS's) for 800 msec prior to delivery of a 100 msec electric shock (3 ma) to the paraorbital region of the head, which served as the unconditioned stimulus (UCS). Effects of 6-HDA were determined by the frequency of conditioned responses (CR's) over the 10 daily sessions and by the number of trials required to reach a criterion of 1, 5, or 10 consecutive CR's. 6-HDA retarded acquisition of the conditioned nictitating membrane response (NMR) to both tone and light CS's under all doses examined with greatest retardation at the 670 µg dose. At this high dose, telencephalic norepinephrine was reduced 89%, dopamine 45%, and serotonin 42% relative to controls. The unexpected serotonin depletion was a consistent finding in these experiments with 18% and 29% depletions at the 270 and 540 µg doses, respectively. Unpaired control studies revealed that the effect of 6-HDA was not mediated by a change in amplitude of the unconditioned NMR.

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87.2 OPIATES AND CLASSICAL CONDITIONING: SELECTIVE ACTION ON CONDITIONED RESPONSES BY CENTRAL ACTIVATION OF MU RECEPTORS. M. D. Mauk, J. Madden IV*, J. D. Barchas, and R. F. Thompson. Depts. of Psychology and of Psychiatry and Behavioral Sciences. Stanford Univ., Stanford, CA 94305

We have previously reported that IV administation of morphine produces selective, naloxone-reversible abolition of classically conditioned responses in rabbits (Mauk et al. Science, 216:434, 1982). We now provide evidence that this selective opiate action on conditioned responses (CR) is mediated by activation of opiate receptors within the central nervous system. Further, highly selective activation of mu receptors is sufficient to abolish CRs.

Morphiceptin (Try-Pro-Phe-Pro-NH_2), an amide derivitive of B-casomorphine-4, has been shown to have highly selective receptor binding to the morphine (mu) receptor. (Chang et al. Science, 212:75-77, 1981). We have observed that infusion of 200nM of morphiceptin into the fourth ventricle via permanently implanted cannula produces pronounced abolition of CR which is blocked by concomitant infusion of naltrexone (20nM). Administation of an inactive isomer of morphiceptin ($D\text{-}Pro^2$) had no effect. Chang and collegues (1982) have reported a potent morphiceptin analog [Try-Pro(NMe)-Phe-D-Pro-NH_2] which retains high binding affinity and selectivity for mu receptors. Central infusion of as little as 12nM of this analog produces long-lasting abolition of CR which is dose-dependent and naloxone reversible. In all cases, the opioid action was selective to the CR, unconditioned responding was unaffected. In sum, the action of morphiceptin and its analog on CRs is dose-dependent, blocked and reversed by antatgonists, and is stereospecific.

Evidence that this effect on learned responses is mediated by central action is supported by two additional results. First, IV administration of the morphiceptin analog in doses ranging 0.1 to 10 times those effective via central injection did not effect the CR. Second, quarternary naloxone, which does not cross the blood-brain barrier, had no effect on morphine induced abolition of CR, even at doses 100 times an equimolar dose of naloxone which completely reverses the effect of morphine.

Collectively, these obserations are consistant with the position that these selective effects of opiates on learned responses are mediated by activation of mu receptors within the central nervous system.

87.3 CENTRAL OPIATE EFFECT ON HEART-RATE CONDITIONING, D.G. Lavond*, M.D. Mauk, J. Madden, IV*, J.D. Barchas and R.F. Thompson. Depts. of Psychology and of Psychiatry and Behavioral Sciences, Stanford Univ., Stanford, CA 94305.

Gallagher and her colleagues (M. Gallagher, B.S. Kapp, C.L. McNall and J.P. Pascoe, Pharmac. Biochem. Behav., 14:497, 1981) have demonstrated that activation of opiate processes within the central nucleus of the amygdala can impair acquisition of classically conditioned heart-rate deceleration in the rabbit. We now report that microinjections of an opiate agonist into the fourth ventricle abolishes conditioned heart-rate decelerations.

Rabbits were initially exposed to 15 tone-alone presentations (1 KHz, 85 dB, 5 sec., 60 sec. ITI) in order to habituate the bradycardia to a novel stimulus. They were then given 15-20 paired trials where the tone was followed by .5 sec. of face shock (2mA, 60 Hz). Such pairings resulted in conditioned heart-rate decelerations to tone onset and an acceleration of heart-rate following shock offset. The animals were matched for learning performance (percentage of heart-rate during the 5 sec. tone divided by the heart-rate during the preceding 5 sec.). Each animal received a microinfusion into the fourth ventricle of either 12 nM of the opiate morphiceptin (Tyr-Pro-(NMe)-Phe-D-Pro-NH_2), a highly selective mu receptor agonist (K.J. Chang, personal communication), or of a cocktail of the methylated morphiceptin and 10 nM of the opiate antagonist naltrexone. Injections of the methylated morphiceptin eliminated the conditioned bradycardia to the tone but not the acceleration to the shock. Rabbits given the cocktail continued to show the same preinjection level of conditioned heart-rate decelerations in the next 5 paired trials. These effects are consistent with the hypothesis that the opiate effect on nictitating membrane conditioning is due to an attenuation of conditioned fear as expressed by the conditioned deceleration of heart-rate.

87.4 DIFFERENTIAL AMNESTIC EFFECTS OF CO_2 AND N_2 ON AVERSIVE AND APPETITIVE TASKS. C.A. Boast and Y. Islami*. Res. & Dev. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

It is commonly recognized that hypoxic conditions can impair memory processing in a time-dependent way. However, previous reports have suggested that the manner in which hypoxia is achieved will determine whether or not amnesia is observed. For example, hypoxia induced by exposure to CO_2, but not N_2, resulted in performance impairments in both single trial inhibitory avoidance (Taber & Banuazizi, Psychopharmacologia 9:382, 1966) and delayed spatial alternation (Marriott & Voightman, Neurosci. Abstr. 6;170, 1980). We have examined this phenomenon in more detail by assessing the amnestic effects of 100% CO_2 or 100% N_2 in both an aversively motivated single trial inhibitory avoidance task and an appetitively motivated single trial maze task.

In the inhibitory avoidance task, animals received a footshock (0.4 mA mice, 1.2 mA rats) upon entering a large dark chamber. Retention was assessed 24 hr later by measuring the latency for each animal to re-enter the dark chamber. Long latencies indicated good retention, short latencies indicated amnesia. In the appetitive task, water deprived (40 hr) animals were placed in an open field having a blind alley in one corner. During the training trial animals explored the maze until they found and drank water located in this blind alley. Retention was assessed 24 hr later by measuring the latency to return to the water. Short latencies indicated good retention, long latencies indicated amnesia.

We found that CO_2 exposure (25 sec mice, 55 sec rats) immediately following training impaired inhibitory avoidance performance in both species, while N_2 (60 sec mice, 90-120 sec rats) had no effect on performance of this task. In contrast, CO_2 exposure had no effect on retention in the appetitive task, but N_2 impaired retention in both species. CO_2, but not N_2, impaired inhibitory avoidance performance of water deprived mice. Thus, water deprivation per se was not responsible for the double dissociation of CO_2 and N_2 effects on these aversively and appetitively motivated tasks.

We conclude that different behavioral experiences initiate different memory processes. For example, limbic structures have been associated with emotionality, while the hypothalamus has been associated with basic physiological drives. Therefore, the aversively motivated task may involve more limbic information processing, while the appetitively motivated task may involve more hypothalamic information processing. These different memory processes may have characteristic neuronal metabolic requirements. It remains to be determined whether these anatomical regions are differentially sensitive to metabolic disturbances by CO_2 or N_2.

87.5 ROLE OF THE DOPAMINERGIC SYSTEM AND CAUDATE NUCLEUS IN SHORT TERM MEMORY. D. G. Cook* and R. P. Kesner (SPON: M. E. Ellis). Dept. of Psychology, University of Utah, Salt Lake City, UT 84112.

A large body of experimental evidence has implicated the caudate nucleus (CN) in learning and memory. In order to elaborate further on the nature of CN nucleus involvement, male Long-Evans rats were trained in a discrete delayed alternation task.

The task required the rat, in a two bar choice situation, to press the bar opposite to the one pressed in initiating the trial in order to obtain reinforcement. The time between the initiation of a trial and presentation of the two bars was varied from 0 to 30 sec. Rats trained on this task show a retention function (as inferred from performance) that decays within 30 sec, reflecting a time course interpreted as a short term memory (STM) gradient.

After training rats either received bilateral injections in the CN of 5 ug Kainic acid in 1 ul of phosphate buffered saline or 1 ul of phosphate buffered saline. Compared to control animals the CN lesioned animals showed a disruption of performance at both short (0-2 sec) and long delay (5-30 sec) retention tests.

In other animals 0.05 mg/kg of haloperidol, a dopamine receptor antogonist, or saline was injected i.p. 30 min prior to testing. Compare to saline injections haloperidol had no effect at 0-2 sec delays, but had a facilitatory effect at all the other retention test delays (5-30 sec).

These data may be explained by noting that dopamine normally has a marked inhibitory function upon neurons within the CN. Consistent with this explanation is a previous observation that amphetamine (a drug which has a facilitatory effect on dopamine action) has a disruptive effect on STM. That these effects are primarily due to dopaminergic and not noradrenergic action is supported by additional experiments, in which it was shown that compared to saline injections .5 and 1.0 mg/kg injection of propranolol, a ß-adrenergic receptor antagonist, or 125-250 mg/kg of diethyldithiocarbamate (DDC, a ß-hydroxylase inhibitor) had no effect upon delayed alternation performance.

This study suggests that the CN and dopamine play an important role in the mediation of STM as measured in a discrete delayed alternation task.

87.6 EFFECT OF HOME OR NOVEL ENVIRONMENT ON SUBSEQUENT PASSIVE AVOIDANCE BEHAVIOR IN MICE TREATED WITH ETHANOL. D.L. Colbern* and E.G. Zimmermann. Brain Research Institute and Department of Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.

We have shown previously that mice injected with ethanol immediately after footshock training increase their passive avoidance behavior when tested 24 hours or 5 days later (Substance Alcohol Actions/Misuse, 1:181, 1980). This enhancement of avoidance behavior by ethanol showed diurnal variation, with maximal levels appearing when training and testing occurred during the last 4 light hours of a 12: 12 hr (light:dark) lighting schedule (Alcoholism: Clin. Exp. Res., 5:146, 1981). In these studies, male Swiss-Webster mice, housed 9 or 10 per cage, were returned to their home cage within 10 min after passive avoidance training and intraperitoneal injection of ethanol or saline.

More recent studies have shown that injections of ethanol or saline at various intervals within 1 hr, after footshock training, but not at 90 or 180 min, affect avoidance behavior 24 hr later. This suggests that events which occur in the home cage after training may influence the effect of ethanol on subsequent avoidance behavior. To investigate this possibility, mice were given one-trial of step-through, passive avoidance training (0.1 mA footshock) immediately followed by i.p. injection of ethanol (3.0 g/kg, 15%) or equivolume saline. They were then either returned to their home cage (10 mice/cage) or individually housed in 32 oz drink containers for 90 min before being returned to their home cage. Training was conducted during the last 2 light hours of a 12:12 hr, light:dark schedule. Avoidance behavior was measured 24 hr later.

The results replicated our previous findings in that when mice were returned to their home cage after shock-training and injection with ethanol, avoidance behavior was greatly increased compared to mice receiving saline ($p<.025$). However, when mice spent 90 min in a novel environment immediately following treatment, ethanol did not enhance avoidance behavior compared to saline controls. Failure to observe ethanol-facilitated avoidance was due to a marked attentuation of avoidance behavior in ethanol-treated animals placed in novel environments and was not secondary to increases in avoidance displayed by saline-treated mice. Passive avoidance behavior of mice injected with saline was the same for both post-training housing conditions.

These findings indicate that environmental factors interact with ethanol to modify retention of passive avoidance behavior in mice. The precise mechanism by which ethanol and novelty interact are currently under investigation. (Supported in part by USPHS Grant NIAAA 03513-05).

87.7 Effect of Cognitive Performance Enhancement (CPE) Reference Compounds in a Rat Avoidance Acquisition Procedure. E. Boff*, E. Gamzu, D. Poonian*, and M. Zolcinski*. Dept. of Pharmacology, Research Div., Hoffmann-La Roche Inc., Nutley, New Jersey 07110.

We studied a lever-press avoidance acquisition test using naive rats to evaluate CPE compounds to enhance learning/memory effects. Male Charles River CDF rats (155-175 grams) were administered a test compound and presented with 60 avoidance trials at one minute intervals. Each trial consisted of a 15 second tone followed by 15 second tone + 1 mA electric footshock. Twenty-four hours later, the rats were retested.

Five CNS stimulants (d-amphetamine, caffeine, magnesium pemoline, methylphenidate (MPD), and nicotine) produced a significant increase in avoidance responding at one or more p.o. doses, all but MPD did so dose-dependently. However, doses which increased avoidance responding, generally produced an increase in motor activity. Warner-Lambert's CI-844, reported to enhance learning of passive avoidance (Butler et al., J. Med. Chem., 1981, 24:346-350) significantly increased avoidances at 30 mg/kg i.p. On the following day (non-drug), there were no dose related or significant effects. Similar results were obtained with the stimulants in a procedure that involved an additional escape-only session prior to avoidance testing. The addition of a vibratory stimulus during the tone period, significantly increased avoidance responding, but decreased avoidance sensitivity to the stimulants.

Compounds used in the pharmacotherapy of organic brain syndrome or senile dementia were also evaluated. Naftidrofuryl (30-300 mg/kg), centrophenoxine (3-300 mg/kg) and hydergine (1-100 mg/kg) did not significantly affect avoidance responding compared to control on either day. However, a significant decrease in avoidances was obtained at 30 and 100 mg/kg with vincamine. Avoidances were not increased by physostigmine (0.03-.3 mg/kg i.p.). A significant increase in avoidances following diphenylhydantoin or piracetam were probably due to low control values and was not replicable. Carbamazepine produced a nonsignificant increase at 3 and 10 mg/kg. Neither ACTH 4-10 (0.01-1 mg/kg s.c.) nor aniracetam (Ro 13-5057, 1-100 mg/kg p.o.) significantly increased avoidance acquisition.

This avoidance acquisition procedure does not appear to be a useful primary test for screening CPE agents, since drugs that have shown some utility in treating geriatric patients were inactive. A compound of interest might be exposed to show a dose-dependent increase in avoidances on drug day with minimal stimulation, and a level of performance significantly above control on the following day.

87.8 LONG-TERM AFTEREFFECTS OF CHRONIC ADMINISTRATION OF METHYLPHENIDATE ON LEARNING AND BRAIN CHEMISTRY IN RATS. Corinne Manetto*, Randall Lockwood*, James A. McCaughran, Jr., and Nisson Schechter (SPON: C. Browman), Dept. of Psychology and Long Island Research Institute, SUNY at Stony Brook, New York 11794.

Methylphenidate (MPH) is frequently the drug of choice in treating children with Attention Deficit Disorder with hyperactivity (ADD/HA). Because of its widespread chronic use, it is important to determine the long-term effects of MPH on the developing nervous system. Although many of the side effects associated with chronic MPH administration will normally disappear when the drug is discontinued, it has only recently become evident that the neuronal elements affected by the drug may undergo lasting, if not permanent alterations.[1] In the present report, the effect of chronic treatment with MPH on brain chemistry and learning and other behaviors were investigated in rats.

Four groups of rats were injected (ip.) with MPH or the saline vehicle once daily beginning at 25 days of age and continuing until 65 days of age. Three different experimental groups were used: 0.1 mg/kg, 5.0 mg/kg and 20 mg/kg. 175-205 days after discontinuation of drug treatment, behavioral assessment was begun. Rats were tested in a step-down passive avoidance task.

Animals treated with MPH required significantly fewer trials to acquire the passive avoidance response than did controls. Two weeks following the acquisition of the passive avoidance task these animals were tested in a one-way active avoidance situation. In contrast to their accelerated passive avoidance acquisition, animals treated with MPH required significantly more trials than controls to learn the active avoidance task. Further observations indicated that the animals treated with MPH defecated more throughout avoidance testing, were less responsive to the shock, were more resistant to extinction and moved more sluggishly than the controls. On criterion tests experimentals showed a greater latency of response to the CS than controls.

Following behavioral testing, the brains of experimental and control animals were subjected to neurochemical analysis. There were several indications that the chronic MPH treating during development results in cholinergic abnormalities in the basal ganglia but not in the hippocampus.

The results of this study indicate that chronic treatment with MPH can have long-term effects on brain chemistry and the ability of rats to acquire simple learning tasks. The implications of this work strike a cautionary note with regard to the chronic use of MPH in children. Clearly, clinical studies are necessary to evaluate the long-term aftereffects of MPH in humans.

[1] Skinner, R.D., et al. Neurosci. abstracts, 7, 780, 1981.

87.9 EFFECT OF ACTH AND EPINEPHRINE AND THEIR INTERACTION WITH BETA-ENDORPHIN AND NALOXONE ON MEMORY CONSOLIDATION. I. Izquierdo and R.D.Dias *. Depto. Bioquímica, Inst. Biociências, U.F.R.G.S. (centro), 90000 Porto Alegre, RS, Brasil.

Adult Wistar rats were trained and retested in two different but closely related step-down inhibitory avoidance tasks: § 1 (25 x 25 cm platform, 0.5 mA footshock) and § 2 (7 x 25 cm platform, 0.3 mA footshock). Immediately after training animals received an ip. injection of ACTH 1-24 (0.2 or 2.0 µg/kg), epinephrine HCl (5.0 or 50.0 µg/kg), beta-endorphin (0.1 or 1.0 µg/kg), naloxone HCl (0.4 mg/kg), or a combination of the former two substances plus beta-endorphin or naloxone. Control animals received saline (1.0 ml/kg). All drugs were dissolved in saline. Training-test interval was 24 hr. ACTH and epinephrine caused retrograde amnesia for Task § 1 and retrograde memory facilitation in Task § 2. Beta-endorphin caused amnesia and naloxone caused facilitation in both tasks. In Task § 1, the amnestic effect of ACTH and epinephrine was potentiated by beta-endorphin and reversed by naloxone. In Task § 2 the facilitation caused by ACTH and epinephrine was potentiated by naloxone and reversed and transformed into deep amnesia by the simultaneous administration of beta-endorphin.

These findings support the view that there is an endogenous amnesic mechanism mediated by beta-endorphin (and perhaps other opioids as well), and suggest that this mechanism interacts with others mediated by ACTH and epinephrine in order to prevent memory from being as good as it could be. The conjoint operation of these various mechanisms (which, in the case of ACTH and epinephrine might depend on the aversiveness and/or the difficulty of the task), and possibly others, results in modulation of the early stages of memory in which it becomes consolidated.

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87.10 LESIONS OF THE STRIA TERMINALIS ATTENUATE THE FACILITATORY EFFECT OF EPINEPHRINE ON RETENTION IN AN INHIBITORY AVOIDANCE TASK. K.C. Liang* and James L. McGaugh, Department of Psychobiology, University of California, Irvine, CA 92717, U.S.A.

Posttraining systemic injection of epinephrine has been shown to affect retention. Recently we have found that adrenal demedullation alters the effect of electrical amygdaloid stimulation on retention. The findings suggest a possibility that the amygdala may be involved in the effect of peripheral epinephrine on memory processes. Since the stria terminalis (ST) has been shown to be involved in the memory modulatory function of the amygdala, the present study examined the effect of ST lesions on the retention facilitation caused by peripherally administered epinephrine.

Male ARS Sprague-Dawley rats (60 days old) received bilateral radiofrequency lesions of the ST (ST-) or sham operations (ST+). Two weeks later animals were trained in a one-trial step-through inhibitory avoidance task (0.7 mA, 0.5 sec footshock). Rats serving as non-footshock controls did not receive footshock during training. Immediately after training, rats were injected subcutaneously with saline or one of three doses (0.01, 0.1 and 1.0 mg/kg) of epinephrine. Retention was tested 24 hr later. Retention latencies of various groups are shown in the table. In the ST+ rats, each of the three doses of epinephrine significantly enhanced retention in the footshock groups, while posttraining epinephrine injection had no effect on the performance of the non-footshock groups. Lesions of the ST did not significantly affect retention. However, the lesions attenuated the memory-facilitating effect of epinephrine. Retention latencies of ST- rats given epinephrine were not significantly different from those of ST- rats given saline, and were significantly lower than those of ST+ rats given the same dose of epinephrine. These findings suggest that an intact ST connection from the amygdala, which has been shown to be involved in processing visceral information from the periphery, is essential for the memory facilitatory effect of epinephrine administered peripherally.

Median Retention Latencies (sec) in the Inhibitory Avoidance Task

Epin.	(mg/kg)	Sal	0.01	0.1	1.0
ST+	FS	88.7 (14)	382.0[a](13)	597.0[a](14)	359.8[a](12)
	NFS	10.4 (7)	18.8 (6)	16.2 (6)	17.0 (6)
ST-	FS	158.2 (11)	168.2[b](11)	154.8[b](10)	216.9[c](9)

FS: footshock, NFS: non-footshock, ( ): number of animals
a. different from the ST+/Sal, $p<0.01$; b,c. different from the correspondent ST+/Epi., $p<0.05$, $0.10 < p < 0.05$, respectively.

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87.11 PERIPHERALLY-ADMINISTERED CATECHOLAMINES DO NOT REVERSE RESERPINE-INDUCED AMNESIA FOR A DISCRIMINATED ESCAPE-REVERSAL TASK IN MICE. L. Wichlinski*, T. Palfai* and O. M. Brown* (SPON: J.-T. Cheng) Psychology Research Labs., Syracuse University and Dept. of Pharmacology, SUNY Upstate Med. Ctr., Syracuse, N. Y. 13210.

Peripheral administration of NE (0.5 mg/kg) or DA (50 mg/kg) to mice 15 minutes before discriminated escape-reversal training in a T-maze failed to reverse amnesia caused by reserpine pre-treatment (2.5 mg/kg) two hours prior to the reversal training session. These results contrast with those obtained previously in a passive avoidance paradigm, where either NE or DA at similar doses and intervals successfully reversed reserpine-induced amnesia (Palfai & Walsh, Behav. Neural Biol. 27: 432, 1979). In the present study no effects on retention were seen in mice given NE or DA after pre-treatment with reserpine-vehicle. Also, no effects on acquisition were observed with any of the treatments.

Correlated biochemical analysis using high-performance liquid chromatography (HPLC) with electrochemical detection showed that peripherally-administered NE or DA did not replete whole brain catecholamine levels diminished by pre-treatment with reserpine. In addition, no effects of peripherally-administered NE on central catecholamine levels were found after pre-treatment with vehicle. However, animals given peripherally-administered DA following vehicle pre-treatment showed significantly elevated brain DA (but not NE) levels compared to vehicle-treated controls. This result was unexpected, since DA presumably does not cross the blood-brain barrier. Studies are underway in our laboratory to replicate this finding.

Our behavioral results indicate that the ability of peripherally-administered catecholamines to reverse reserpine-induced amnesia is task-dependent, since reversal of amnesia occurs with catecholamines administered in the passive avoidance paradigm, but not in the escape-reversal paradigm. In addition, reversal of reserpine-induced amnesia with peripherally-administered NE or DA is not accompanied by a repletion of brain catecholamines. Thus, peripheral adrenergic mechanisms appear to play an important modulatory role in some memory tasks, but not others.

87.12 FACILITATION OF HIPPOCAMPAL KINDLING BY PAPAVERINE AND SYNAPTIC MEMBRANE PROTEIN PHOSPHORYLATION. D. L. Chute, J. Gurd*, B. Bank*, N. W. Milgram, S. Mandel*, Div. of Life Sci., Univ. of Toronto, Scarborough, Ontario M1C 1A4 and Dept. of Psychiatry, McMaster Univ., Hamilton, Ontario.

The drug papaverine, a phosphodiesterase inhibitor, functionally increases levels of cAMP. Post-trial injection of this drug has been found to improve memory for a variety of types of retention tasks. These results suggest that cAMP dependent protein phosphorylation may be the underlying mechanism for learning. A hypothesized model for learning is the kindling phenomenon. Kindling of limbic and forebrain structures occurs with the daily application of an electrical stimulation of sufficient intensity to trigger afterdischarges. Eventually, behavioural seizures evolve. We show kindling is facilitated with 50 mg/kg (but not 25 mg/kg) of papaverine. Rats were kindled daily in the CA3 region of the hippocampus and were injected I.P. with papaverine or vehicle control either immediately before receiving the kindling stimulus or 1 hr. prior to kindling. The results indicate that (50 mg/kg) papaverine injected at the time of a 60 Hz., 1 sec. train of electrical brain stimulation does enhance rates of hippocampal kindling. Animals receiving contingent papaverine exhibited generalized seizures after only 14 days as compared to 28-42 days for controls. Synaptic membranes were isolated from the rat hippocampi, labeled with [γ-^{32}P] ATP and the extent of its incorporation in synaptic proteins and glycoproteins determined.

87.13 MONOAMINERGIC ANTAGONISTS AND SPATIAL WORKING MEMORY IN RATS. William W. Beatty and James R. Rush*. Department of Psychology, North Dakota State University, Fargo, ND 58105.

When tested in the 8 arm maze spatial working memory in rats is remarkably long lived and resistant to retroactive interference. Normal forgetting consists mainly of repetitions of choices made many hours before and only rarely includes repetitions of choices made during the retention test (retention errors). Because of its longevity spatial working memory is an ideal test for assessing the effects of biological treatments on working memory since they can be applied during the retention interval after the to-be-remembered event. Previous work (Shavalia, et al., Behav. Neural. Biol. 1981, 31, 269) indicates that ECS disrupts spatial working memory in a time-dependent way that resembles natural forgetting. To provide an initial assessment of the neurochemical mechanisms underlying spatial memory we examined the effects of several receptor antagonists varying both dose and time of administration within the retention interval. Propanolol (10-20 mg/kg), phentolamine (5-20 mg/kg) and methysergide (5-15 mg/kg) had no effect on spatial memory. Scopolamine (1-5 mg/kg) modestly impaired retention, but the effect appeared to result from a change in performance. Only haloperidol (0.25-1 mg/kg) interfered with retention in a time-dependent way that resembled natural forgetting. Studies with naloxone, currently in progress, will be reported as well.

87.14 MEMORY AND THE SEPTO-HIPPOCAMPAL CHOLINERGIC SYSTEM IN THE RAT. B.J. Davis*, G.N.O. Brito, L.C. Stopp*, and M.E. Stanton.* (SPON: W.E. O'Neill). Center for Brain Research, University of Rochester School of Medicine & Dentistry, Rochester, NY 14642.

This study examined the effects of intra-hippocampal injections of scopolamine on performance of contingently reinforced T-maze alternation (working memory) and visual discrimination (reference memory) by the same rats in the same maze. In experiment 1, rats (N=12) were trained to perform T-maze alternation (10 sessions of 13 trials, 1 session per day) and then received bilateral implantations of cannulas aimed at the CA2-CA3 field of the dorsal hippocampus. Following a 12-day postoperative recovery period and 4 additional sessions on T-maze alternation training, the rats received, 15 min before testing, 1 µl injections of scopolamine (35 µg/µl) or saline alternately for 4 days. Rats were then trained (under no drug) on a visual discrimination task in the T-maze (20 sessions of 12 trials, 1 session per day) and then tested under drug (or saline) as described above.

In experiment 2, rats (N=11) were trained to perform the visual discrimination task first. Following surgery, a 10-day postoperative recovery period, and 3 additional sessions of discrimination training, the rats received intra-hippocampal injections of scopolamine (1 µl) at three different concentrations (35, 12, and 4 µg/µl) on different days alternated with saline injections. The rats were then trained (under no drug) on T-maze alternation and tested under the drug conditions as described for the visual discrimination task.

Experiment 1 showed that scopolamine impaired performance of both tasks relative to saline injections which had no effect. However, the impairment induced by scopolamine was much larger on T-maze alternation than on visual discrimination. Experiment 2 replicated this finding with the order of the tasks reversed and, additionally, showed that T-maze alternation was impaired at both the 35 and 12 µg/µl doses whereas visual discrimination was impaired only at the 35 µg/µl dose. A dose of 4 µg/µl had no effect on either task.

We conclude that performance of a working-memory task is significantly more sensitive to intra-hippocampal injections of scopolamine than performance of a reference-memory task.

87.15 THE EFFECTS OF D-LYSERGIC ACID DIETHYLAMIDE (LSD) ON NEOPHOBIA AND EXPLORATORY ACTIVITY IN RATS. L.M. Adams* & M.A. Geyer. Dept. of Psychiatry, Univ. of Calif., San Diego, La Jolla, CA 92093.

While LSD has been shown to reduce rates of habituation to discrete phasic stimuli, open field studies ("forced-exploration") have typically revealed reduction in locomotion which have been interpreted as reduced sensitivity to the environment. Since our work with "forced-exploration" tests indicated that LSD's suppression of locomotion might be attributable to a potentiation of neophobia (Geyer, M.A. and Light, R.K., Psychopharmacology 65:41, 1979), the present experiments utilized a new computerized pattern/activity monitor which included a homecage attached to a holeboard chamber, allowing the rats to explore the novel chamber at will ("free-exploration"). Male Sprague Dawley rats (275-300g) were injected (s.c.) with 30 µg/kg LSD or saline either 0 or 20 min prior to being placed in the homecage. Ten min later, a door opened, permitting entry into the 12x24 in. holeboard where crossovers, rears, and holepokes were monitored. Routes of locomotion were reconstructed from the stored sequences of X-Y position changes. During the first half of a 60 min session, LSD-treated rats made fewer entries into and spent less time in the novel chamber than controls, followed by a steady increase in both measures from 30-60 min. The time-course of this effect was independent of pre-injection time. Although LSD's initial reduction of time spent in the holeboard resulted in lower overall levels of crossovers, rears and holepokes, examination of the ratio of these measures to the time spent in the holeboard revealed no group differences in rates of activity. Further, LSD rats failed to establish the stereotyped excursion routes (round-trips between the homecage and various parts of the holeboard) characteristic of controls. Rather, their excursions were meandering and seldom retraced, suggesting a failure to adapt to the novel environment. Thus, it seems unlikely that LSD's initial suppression of holeboard exploration reflects a lack of curiosity or non-specific supression of locomotion, but instead reflects a potentiation of the initial neophobia exhibited by controls. In a subsequent dose-response study, 30 µg/kg LSD produced the same effect seen in the previous time-course study. Eighty µg/kg LSD extended the period of neophobia to 40 min but still had no effect on activity rates, while 8 of 10 rats in the 160 µg/kg group never entered the holeboard, indicating a dose-dependent potentiation of neophobia. In contrast, 10 µg/kg LSD increased the number of entries into and time spent in the holeboard, particularly during the first 10 min. We are presently examining the locomotor patterns to determine whether the 10 µg/kg effect reflects a reduction of neophobia or an enhancement of investigatory responding made apparent by the absence of potentiated neophobia.

87.16 DRUG-INDUCED MEMORY IMPAIRMENTS IN NON-HUMAN PRIMATES. R. L. Dean B. Beer and R. T. Bartus. Dept. CNS Research, Medical Research Division, American Cyanamid, Lederle Labs, Pearl River, NY 10965

In a series of studies, we tested several drugs in an attempt to ascertain their specific memory-impairing properties. Cebus monkeys were run on a delayed response task which required the monkeys to push the one panel (of nine), which had been previously illuminated. The opportunity to push the panel occurred either immediately after the panel was extinguished (zero-sec delay) or after a forced delay (delayed retention interval). Operationally, a drug was defined as impairing memory when a significant decrease in accuracy occurred on the delayed retention interval (when location of the stimulus had to be remembered for a predetermined period before the opportunity to respond occurred), without a concurrent effect on the zero-sec condition (when the monkey could respond as soon as the panel was extinguished). In this way, it was possible to determine whether a drug selectively impaired performance when recent memory was required, without simultaneously impairing the more general performance aspects of this task when little recent memory was needed.

Clear differences existed between the ability of various drugs to induce memory impairments on this task. Of the drugs tested, THC produced the greatest selective impairment. Other drugs which selectively impaired performance were the anticholinergic scopolamine HBr and atropine sulfate, the tricyclic antidepressant amitriptyline, the anxiolytic diazepam and sedative hypnotic pentobarbital. No selective impairment was observed for the quarternary anticholinergic scopolamine MBr, the centrally-acting nicotinic antagonist, mecamylamine, the dopaminergic antagonist, haloperidol, or the tricyclic antidepressant, desimpramine.

These data will be discussed as they relate to (a) similar effects reported by others, with humans, (b) predicting the amnestic properties of new drugs using an animal model, (c) helping to characterize some of the pharmacological and neurochemical mechanisms involved in drug-induced memory loss.

87.17 SHORT-TERM MEMORY IN THE MONKEY: A MATCH-TO-SAMPLE PROCEDURE WITH VARIABLE RETENTION INTERVALS AND INITIAL STUDIES OF d-AMPHETAMINE, PHYSOSTIGMINE AND PIRACETAM. E. Schwam*, L. Rumennik*, A. Kuehn*, and J. Sepinwall. Dept. of Pharmacology, Hoffmann-La Roche Inc., Nutley, NJ 07110

As part of a program to develop pharmacologic agents to treat human cognitive dysfunctions, we previously described a delayed match-to-sample procedure in squirrel monkeys and the effects of various reference compounds (Schwam et al., Neurosci. Abstr., 1981, 7:526). This procedure was limited to studying the effects of compounds upon a single level of retention.

Eleven adult male squirrel monkeys have been extensively trained on a modification of our original procedure. As before, each trial consisted of two successive components. First, animals were required to select a choice stimulus that matched a simultaneously presented sample stimulus. Following two consecutive correct matches all keys were darkened for a selected delay interval after which only the three choice keys were illuminated. Responding to the correct choice key after the delay interval resulted in food delivery. Five delay intervals extending from 0-95 sec were preselected for each animal and presented in each session according to a randomized blocks sequence. The delay values were chosen to achieve performance levels ranging linearly from near perfect to chance. After stable baselines were established, d-amphetamine sulfate (0.025-0.4 mg/kg i.g.), physostigmine salicylate (0.01, 0.02, 0.04 mg/kg i.m.) and piracetam (400, 800, 1600 mg/kg i.g.) were evaluated for their ability to enhance memory. Acute administrations of each dose were given at weekly intervals.

Analyses of results across delay intervals demonstrated significant increases in retention at one or more dose levels of each compound. These improvements were, however, of small magnitude - no greater than 15% above control. In addition, adverse effects were obtained at the highest dose levels tested of d-amphetamine (cessation of responding and failure to complete all scheduled trials) and physostigmine (cholinergic side effects). Results were also examined as a function of retention interval for each compound. The maximum improvements above control were as follows: d-amphetamine, 39% for the longest delay interval at 0.05 mg/kg; physostigmine, 28% for the fourth longest delay interval at 0.02 mg/kg; piracetam, 23% for the third longest delay interval at 400 mg/kg.

While instances of enhanced retention were seen with each of the compounds tested, improvements were of limited magnitude. These findings are consistent with the equivocal efficacy and side effect liability of available drugs applied to the treatment of cognitive dysfunctions. Considering the number of affected patients and severity of symptoms, the need to develop novel compounds with more dramatic effects is emphasized.

87.18 EFFECTS OF NICOTINE ON HABITUATION IN HUMANS: A PSYCHOPHYSIOLOGICAL STUDY. S. Servidio and G.G. Berntson. Lab. of Comparative and Physiological Psychology, Ohio State Univ., Columbus, OH 43212

The present study examined the effects of nicotine on stimulus reactivity and habituation as measured by the cardiac orienting response. The paradigm consisted of repeated presentations of two auditory stimuli presented in close temporal sequence. Interspersed within these trials were dishabituation trials, in which the first stimulus was presented and the second stimulus withheld.

A high nicotine cigarette (1.34 mg) was found to appreciably affect both orienting and habituation relative to a no smoking control condition or low nicotine cigarette (.13 mg). At the high dose, nicotine produced an enhancement of the orienting response to the auditory stimuli, as evidenced by increased cardiac deceleration on the initial trials. This finding indicates that nicotine can enhance sensory reactivity. Moreover, the enhanced cardiac orienting response under the high nicotine condition was much slower to habituate, suggesting that nicotine may also facilitate the maintenance of attention to sensory stimuli. These findings are consistent with previous reports that nicotine can raise the visual critical fusion frequency, can increase reaction time efficiency, and can facilitate attentional performance in some paradigms.

These data may offer insight into the psychological and behavioral effects of nicotine. The facilitation of sensory reactivity and attention to external stimuli may account for the actions of nicotine in complex tasks of performance and learning.

88.1 OBSERVATIONS ON INTRACRANIAL PRESSURE (ICP) IN THE RABBIT. T.J. Malkinson*, W.L. Veale, and K.E. Cooper. Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

ICP was continuously measured in adult male New Zealand White Rabbits by means of the Subarachnoid Screw Technique. Data represents the maximum Mean change and the Standard Error of the Mean from a group of six animals. ICP is expressed in mm.saline.

Experiments in the anesthetized animal examined the ICP change resulting from various rates of volume infusion into the lateral cerebral ventricle as well as the change in ICP resulting from the intravenous (IV) administration of Pitressin. Results obtained for volume infusion were similar to those obtained by other investigators--that of rapidly increasing ICP as compensatory mechanisms are exhausted. Administration of Pitressin resulted in a short duration increase in ICP; 0.23 units/kg 18.89 (0.83)mm, 0.05 units/kg 9.47(1.26)mm.

In unanesthetized animals IV infusion of 100 mg/kg/min urea over 15 minutes resulted in a fall of 45.70(4.50)mm. Bolus IV administration of epinephrine resulted in a dose dependent rapid rise in ICP; 10.0ug/kg 55.70(5.91)mm, 5.0ug/kg 25.46(0.85)mm, 2.5ug/kg 17.33(1.35)mm, 1.25ug/kg 11.81(1.27)mm.

In one group of six unanesthetized animals ICP was measured before and after administration of 0.025ug/kg of the Bacterial Pyrogen extracted from *Salmonella abortus equi*. This resulted in an increase in rectal temperature of 1.84(0.11)°C, an increase in mean ICP of 14.30(3.01)mm, a fall in pulsation rate of 63.50 (5.21)%, and an increase in pulsation pressure amplitude of 11.86 (2.02)mm.

In another group of six unanesthetized animals ICP was measured by means of direct cannulation of the lateral cerebral ventricle before and after administration of 0.0075ug/kg of the same Bacterial Pyrogen. This resulted in an increase in rectal temperature of 1.02(0.52)°C, an increase in mean ICP of 19.86 (4.77)mm, a fall in pulsation rate of 50.20(8.21)%, and an increase in pulsation pressure amplitude of 17.21(2.41)mm.

One major influence upon ICP is that of venous pressure. Central venous pressure was measured by direct catheterization in a group of six unanesthetized animals before and after administration of 0.025ug/kg of the same Bacterial Pyrogen. The mean venous pressure rose slightly 6.04(0.64)mm during the initial rising phase of the fever, and then fell to control or less than control values during the remainder of the fever. Body temperature rose 1.67(0.08)°C.

The above results were significantly different from matched control experiments. They demonstrate the influence of these agents upon ICP, and suggest a slight rise in ICP during the "chill" phase of a fever. (Supported by the MRC of Canada)

88.2 IMMUNOREACTIVE LHRH-FIBERS ARE INTIMATELY ASSOCIATED WITH TANYCYTES. P.W. Coates and G.P. Kozlowski. Dept. of Anatomy, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430 and Dept. of Physiology, Univ. of Texas Health Sciences Center, Dallas, TX 75235

A close ultrastructural relationship between luteinizing hormone - releasing hormone (LHRH)-fibers and basal processes of tanycytes coursing in the palisade zone of the median eminence (ME) towards the portal vasculature has been proposed (Kozlowski and Hostetter, Brain-Endocrine Interaction III:138-153, 1978). We now report the intimate relationship between immunopositive LHRH-fibers with tanycyte cell bodies of the third ventricle (3V), using correlative light and transmission electron microscopy (LM and TEM). Sections from brains of adult male Long-Evans rats were prepared for the immunocytochemical localization of LHRH using a pre-embedding procedure previously described (Kozlowski, et al., Peptides 1: 37-46, 1980). Solid-phase immunoabsorption of the rabbit anti-LHRH (No. U-705 and 706) using synthetic LHRH abolished staining. Some sections were retained for LM evaluation. Others were embedded in resin for TEM analysis. LM examination of the sections showed the expected clear delineation and concentration of beaded immunopositive LHRH-fibers sweeping ventrolaterally on both sides of the ME in the region of the tuberoinfundibular sulcus. The LHRH-fibers coursed in parallel with tanycyte processes as they descended to the portal vessels. Some LHRH-fibers closely approached the lumen of the 3V. TEM revealed numerous examples of extremely small diameter (< 1.0μm) unmyelinated LHRH - containing fibers intimately associated with the cell bodies of tanycytes. Profiles of such fibers were observed both in between the lateral cell borders of tanycytes lining the lumen of the 3V, and also apposed to the cell membranes at the basal border of tanycyte cell bodies. Within immunostained LHRH-fibers, aggregates of final reaction product were associated not only with neurosecretory granules but also were dispersed in the axoplasm as well. The varicose LHRH-fibers of the ME might be synaptically linked to tanycytes via such varicosities, forming an as yet undetermined functional relationship. Alternatively, LHRH-fibers may be merely using tanycyte processes and cell bodies as a morphologic substrate for coursing towards the portal vasculature.

Supported by grants from the NIH: HD-12781 and HD-15040 (GPK) and HD-12833 (PWC); and a grant from the Institute for Biomed. Res., TTUHSC (PWC).

88.3 DIFFERENTIAL SENSITIVITIES OF PLASMA AND CEREBROSPINAL FLUID VASOPRESSIN TO OSMOTIC CHANGES IN THE RAT. A. Negro-Vilar and R.S. Kiser. Departments of Physiology and Psychiatry, The University of Texas Health Science Center, Dallas, Texas 75235.

Vasopressin is found in both blood and cerebrospinal fluid (CSF). Serum vasopressin levels are known to rise in response to conditions of increased blood osmolarity, such as water deprivation and administration of hypertonic saline. Less is known about the sensitivity of CSF vasopressin to osmotic changes. The goal of this study was to characterize CSF vasopressin immunoreactivity and to compare the responses of CSF and serum vasopressin to osmotic stimuli such as water deprivation or hypertonic saline administration.

Ten adult male rats were implanted with chronic cisterna magna cannulae. Daily CSF samples were collected and pooled for chromatography on a Sephadex G-25 column. Collected fractions were assayed for arginine vasopressin (AVP) immunoreactivity using a highly specific antiserum. The results revealed a single peak eluting at the position of AVP. Another group of animals was prepared with both cisternal and jugular cannulae. Blood and CSF samples were taken prior to and after a 48 hr period of water deprivation. Assay of samples for AVP immunoreactivity revealed that water deprivation resulted in large increases in serum AVP levels, whereas CSF levels were unchanged. We also compared the effects of intraperitoneal injections (1.1 ml/100 g body weight) of either hypertonic (4.5%) or normal (0.9%) saline. Serum and CSF samples were taken 20 minutes after the injections. Hypertonic saline produced elevations in serum but not CSF AVP levels, when compared to normal saline. To determine whether the CSF response to hypertonic saline could be a delayed one, CSF samples were taken 40 minutes, rather than 20 minutes, after the hypertonic treatment. Again, no change in AVP levels in CSF was detected.

These results show that, unlike serum AVP, CSF levels of the peptide are unaffected by osmotic stimuli, indicating a possible independent regulation of AVP release to the two compartments. Further, they suggest that AVP in CSF may be related to central functions other than those concerned with osmoregulatory mechanisms.

This research was supported by a grant from Smith-Kline and French.

88.4 GLIAL FIBRILLARY ACIDIC PROTEIN IMMUNOREACTIVITY IN THE SUBCOMMISSURAL ORGAN AND MEDIAN EMINENCE OF THE HAMSTER. Daniel B. Michael and James S. Hatfield*. Dept. of Anatomy, Wayne State University School of Medicine, Detroit, MI 48201.

Glial fibrillary acidic protein (GFAP), the major component of glial intermediate filaments, is considered to be a marker peculiar to astrocytes or cells of astroglial lineage. The indirect immunoperoxidase technique of Nakane was used to visualize structures immunoreactive for GFAP in 10 μm frozen transverse serial sections of female golden hamster brains. Specific antiserum to GFAP was supplied by Dr. Lawrence Eng, Veterans Administration Hospital, Palo Alto, CA. Controls included the use of non-immune rabbit serum and GFAP-absorbed anti-GFAP as primary antisera. Both fibrous astroglia of the nerve tracts and protoplasmic astroglia of the neuropil were highly immunoreactive, presenting a stellate appearance similar to that seen with Golgi stains. The perikarya and processes of tanycytes along the third and lateral ventricles and in the median eminence also exhibited GFAP-immunoreactivity, in accordance with previous reports. The floor of the third ventricle at the level of the median eminence contained a prominent round midline mass of GFAP-immunoreactive perikarya and processes. This "glial tangle", extending about 100 μm along the ventricular floor, is thought to be continuous with the supraependymal cluster, previously described by Card and Mitchell (Card, J.P., Mitchell, J.A., J. Comp. Neurol., 180: 43, 1978), and may constitute its site of attachment to the ventricular surface. The structure is not present in similar brain sections from mice or rats. The horseshoe-shaped subcommissural organ (SCO) is composed of thickened columnar ependymal cells at the dorsal tip of the third ventricle. Interposed between these cells are narrow GFAP-immunoreactive perikarya whose bipolar processes extend radially throughout the walls of the SCO. The appearance of these immunoreactive cells, presumably glial, in the median eminence and SCO may provide insights concerning the functions of these circumventricular structures.

88.5 HORSERADISH PEROXIDASE (HRP) INJECTED IN THE CEREBROSPINAL FLUID (CSF) OF THE DOG SHOWS A PREFERENTIAL PENETRATION IN THE VENTRAL BRAINSTEM. C.L. Chernicky, K.L. Barnes, L. Michelini* and C.M. Ferrario. Cleveland Clinic Research Division, Cleveland, OH 44106.

We have studied the distribution of horseradish peroxidase following its injection either into a lateral cerebroventricle (IVT), the cisterna magna or intravenously (IV). Twenty to 30 minutes after CSF injection of either 0.1 or 0.15 ml of 0.5% HRP (Sigma VI) the heads of six anesthetized (morphine-pentobarbital) dogs were perfused via the ascending aorta with 0.9% saline, followed by 5% glutaraldehyde and a final wash of cold 10% sucrose buffer. Serial sections from C2 through the rostrum of the corpus callosum were cut at 50 µm; every 5th section was processed for HRP histochemistry using tetramethyl benzidine as the chromagen. Characteristically, cisterna magna injections caused maximal penetration of HRP into the parenchyma via the ventral surface of the brain extending from the spinal cord to the preoptic area. Label was seen in all ventricles but was restricted to the ependymal layer. On the other hand IVT HRP penetrated both the dorsal and ventral surfaces of the brainstem; HRP reaction product was visualized extending at least 1 mm from the ventricular surface. The corpus callosum was labeled intensely only after cisterna magna injection. In both conditions HRP appeared to follow the perivascular and perineural routes to enter the brain parenchyma from the ventral surface. Cortical tissue was minimally labeled by either route. HRP-filled tanycytes were seen in the organum vasculosum of the lamina terminalis, the area postrema (AP) and the dorsal motor nucleus of the vagus after either cisterna magna or IVT injection. There was diffusion of HRP label into the surrounding medulla via the perivascular spaces; this phenomenon was not observed in the AP. Control injections of HRP into either a jugular vein or cortical tissue did not reproduce the effects observed after injection of the enzyme via the cisterna magna or IVT route. These data indicate that: 1) proteins transported by either ventricular or cisternal CSF reach the entire ventral surface of the brainstem; 2) functional evaluation of neuropeptides given into the ventricular CSF must take into account their possible effect upon structures located in the ventral brainstem. (Supported by grants from NHLBI [HL-6835] and the Reinberger Foundation).

89.1 ASYMMETRIC OLFACTORY MIGRATORY STREAM GROWTH IN THE RAT. W.A. DeBassio*, T.L. Kemper* and A.M. Galaburda (SPON: M. Mesulam). Neurological Units of the Boston City Hospital, Boston 02118 and Beth Israel Hospital, Boston 02215 and the Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The cerebral cortex of human and non-human species is asymmetrical. Thus certain regions can be larger on one side (Galaburda et al, Science 199:852, 1978). Furthermore, cortical folding during development proceeds at different rates on the two sides, with the left side folding later in some areas (Chi et al, Ann. Neurol.1:86, 1977). Although developmental mechanisms underlying these asymmetries remain largely unknown, right-left differences in patterns of cell migration may play a role. In the rat the subependymal layer of the anterior lateral ventricle generates cells that migrate along a well defined stream to the internal granular layer of the olfactory bulb. The present study was undertaken to define growth parameters of this migratory stream in search of possible side differences.

An asymmetry was noted in the growth schedule of the migratory stream. Nissl stained sagittal serial brain sections from rats 10, 30 and 90 days of age were projected at a fixed magnification and the length of the migratory stream was measured. This length increased on the left from a mean of 4.7 mm to 6.38 mm to 7.78 mm at 10, 30 and 90 days respectively. On the right the increases were from 4.8 to 7.09 to 7.73 respectively. Significant growth occurred on the left when the 10-day group was compared to the 30+90-day groups ($F=21.3$; $p<.05$) and when the 30-day group was compared to the 90-day group ($F=5.7$; $p<.05$). On the right side, however, there was significant growth between the 10 and 30+90 groups ($F=25.7$; $p<.05$) but not between 30 and 90 ($F=1.2$). There were no significant side differences in the actual length of the stream at any given age.

The findings of this study show that the development of brain asymmetries may begin as early as the period of cell migration. Thus during early migration to the olfactory bulb, the migratory stream grows more slowly on the left side. Despite this growth asymmetry the actual length of the stream shows no lateral differences at a given age, thus stressing the dynamic nature of the asymmetry. These data are consistent with other evidence suggesting the slower growth of the left hemisphere (see above). Furthermore the findings emphasize the need to control for laterality in studies of neuronal migration. (Supported by NICHD grant 5 PO1 HD 06364 and NINCDS grant 2 RO1 NS 14018).

89.2 AN ANATOMICAL STUDY OF THE DEVELOPMENT OF THE SEPTO-HIPPOCAMPAL PROJECTION IN THE RAT. T.A. Milner and D.G. Amaral Dept. of Neuroscience, UCSD and The Salk Institute, La Jolla, CA 92093

Since the septo-hippocampal projection is thought to be cholinergic, previous studies of its development have used acetylcholinesterase (AChE) histochemistry to determine the time of arrival of the septal fibers in the hippocampus. There is still some uncertainty, however, as to whether the presence of AChE-staining in the developing hippocampus is a reliable indicator of the initial ingrowth of the septal fibers or whether it reflects some later facet of their functional or metabolic maturation. We have, therefore, compared the developmental pattern of AChE-staining within the hippocampal formation with the time of appearance of the septal projection to the hippocampus using anterograde labeling of the fibers with ^{3}H-proline autoradiography, and retrograde labeling with either HRP or the fluorescent dye fast blue, after injections on fetal days (FD) 20 and 21, on postnatal days (PD) 0 through 21 and in adults; in each case the post injection survival time was 6 hours.

Our findings indicate that the septo-hippocampal projection is established at least by FD20. At this time, labeled cells can be seen in the medial septal and diagonal band nuclei following injections of the retrograde tracers into the hippocampal formation. Labeled fibers can be seen in the hippocampal formation following injections of ^{3}H-proline into the septal nuclei on FD21, the initial stage at which orthograde labeling was attempted. The appearance of AChE staining within the hippocampal formation follows the autoradiographically demonstrated pattern of septal fiber innervation by about 5 days. AChE-positive cells can be seen in the hippocampal formation on FD21, but it is not until PD3 that stained fibers are found in the regio inferior of the hippocampus or in the molecular layer of the dentate gyrus at rostral levels. By PD5 AChE-positive fibers are found throughout the rostro-caudal extent of the hippocampus and the dentate gyrus. The adult staining pattern is established by PD14. After injections of ^{3}H-proline into the septal nuclei, diffuse labeling is seen throughout the hippocampal formation between FD21 and PD5. After PD5 the number of silver grains over the strata lucidum and radiatum of regio inferior of the hippocampus and the molecular layer and hilus of the dentate gyrus becomes relatively greater than over the surrounding areas. The adult pattern of innervation appears at PD14. Thereafter as the hippocampus matures, the pattern of AChE-staining no longer closely reflects the pattern of septal innervation.

Our results indicate that the septo-hippocampal projection is established earlier than has generally been recognized and confirm that AChE-staining is an unreliable indicator of the septal input.

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89.3 QUANTITATIVE ANALYSIS OF DENDRITIC DEVELOPMENT IN THE DENTATE GYRUS OF THE MOUSE R.S. Williams, S. Matthysse* & L. Hassinger* E.K. Shriver Center, Waltham, Ma. & McLean Hospital, Belmont, Ma.

In a previous study we used a computer-assisted microscope and Golgi impregnations to define a set of geometric parameters which describe mathematically the class-characteristic size and shape of granule cell dendrites. Size-related parameters include the number of segments, their individual and aggregate lengths. Shape-related parameters include four angles. The daughter angle measures the divergence of subordinate segments at each branch point. The planar, axial and bisector angles measure the spatial relationships of the planes containing the subordinate segments at each branch point with the axis of symmetry of all the dendrites. Granule cell dendrites are typically distributed in the shape of an inverted cone, the axis of which can be defined mathematically.

The present study establishes a family of growth curves for the values of these parameters between postnatal days 7 & 120 (P7-120). Dentate granule cells begin dendritic differentiation during the first postnatal week. By P7 most exhibit the characteristic conical shape, and by P14 most have achieved a measure of axial symmetry comparable to cells at P120. Mean values for daughter and planar angles decline to reach mature values by P21; means for axial and bisector angles change little from P7-P120. Mean values for individual and aggregate segment length vary widely at all ages but increase significantly through P120, with the most rapid phase of growth between P7-P14. Although the range of values for segment number per cell also vary widely, especially at P7 & P14, mean values change surprisingly little between P7-P120. There is a small but statistically significant reduction in mean segment number from P14-P60, followed by a rise at P120. There is also a small but significant reduction in the mean number and variance of stem dendrites arising from the cell soma between P7-P120. Taken together the data confirm that the conical shape and axial symmetry of granule cell dendrites are established before P21. By contrast size-related parameters change continuously through P120. Changes in the mean and variance for segment number confirm that some segments are lost during growth and differentiation, and that the capacity to form new ones is retained through P120. (Supported in part by NIH grants MH34079, P30HD4147 & RSDA K02MH00108)

89.4 RESPONSES TO GABA IN DEVELOPING RABBIT HIPPOCAMPUS. R. M. Chesnut* and P. A. Schwartzkroin (SPON: A. Wyler) Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

Developmental studies in the rabbit hippocampal slice preparation have revealed a lack of inhibitory activity in the neonate despite the existence of an active interneuron population. Anatomical studies have further demonstrated the existence of Gray Type II synapses at this age, as well as the presence of GABA, the putative primary inhibitory neurotransmitter in the adult. We have tested the hypothesis that immaturity in the GABA receptor is responsible for the lack of IPSPs by contrasting adult vs neonatal hippocampal CA1 cell responses to orthodromic stimulation and application of GABA onto the soma.

350-400 μ thick slices were made transverse to the longitudinal axis of the isolated rabbit hippocampus and maintained at 35° C *in vitro*. Intracellular recordings were made in the CA1 region; GABA was applied to the soma via iontophoresis or pressure ejection and synaptic input was elicited via stimulation of the Schaffer collaterals.

In the mature rabbit, orthodromic stimulation elicits an EPSP-IPSP sequence, the IPSP reversing at between -55 to -60 mV. Somatic application of GABA results in either hyperpolarization or a hyperpolarization-depolarization biphasic response. The hyperpolarization reverses at -55 to -60 mV, similar to the IPSP, while the apparent reversal potential for the depolarization is -40 to -45 mV. The immature CA1 cell reacts differently to orthodromic stimulation, showing a long (150-250 msec) depolarization without evidence of an IPSP. Intracellular injection of depolarizing current, however, reveals the biphasic nature of this response, with the second phase reversing at approximately -45 mV and corresponding temporally with the adult IPSP. The response to somatic GABA application is also depolarizing, with a similar apparent reversal potential.

We thus hypothesize that the lack of an IPSP in the immature hippocampus, and its subsequent appearance during development, corresponds to the emergence of a sub-population of GABA receptors associated with somatic inhibitory synapses and mediating their hyperpolarizing responses.

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89.5 DEVELOPMENT OF EMBYRONIC HIPPOCAMPAL TRANSPLANTS IN THE ADULT RODENT CNS. Lawrence F. Kromer. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405

Embryonic tissue from the mid septal-temporal axis of the hippocampal primordia was dissected from embryonic day 17 (E17) rat fetuses (day of mating = E0) and implanted into an intracephalic cavity in adult female Sprague-Dawley rats. The implanted tissue was placed adjacent to the severed edge of the host hippocampal formation on the vascular bed overlying the dorsal thalamus and superior colliculus. After survival times of 1, 8, 15, and 22 days the region of the host CNS containing the transplant was processed to identify the cytoarchitectural organization of the hippocampal transplant during early postimplantation development. At E17, pyramidal cells are starting neurogenesis and there is little laminar organization of neurons in Ammon's horn. Moreover, a granule cell layer also is absent since cells forming the dentate primordium are only beginning their migration from the neuroepithelium. By 1 day posttransplantation the implant is composed of a well defined layer of neuroepithelial cells that contain numerous mitotic figures. Adjacent to one side of this zone are postmitotic neurons that are organized into a loose meshwork of cells. Though there are some pyknotic cells within the implant, its general appearance is similar to that observed for the hippocampal primordium in utero. Some sinusoids are present in the tissue, but there are no direct vascular connections with the host CNS. The transplant is surrounded by cerebrospinal fluid that contains some hemopoietic elements. Even at this early stage, portions of the implant are attached to lesioned surfaces of the host CNS that possess reactive astrocytic processes. By day 8, blood vessels from the host have invaded the implant which has increased in mass. Though a well-defined ependymal zone is absent, some areas of the implant still contain scattered mitotic cells. Additional regions contain immature pyramidal neurons that form loose aggregates or lamina. At 15 days few mitotic figures are present in the implant and both pyramidal and granule cells can be identified. By 22 days posttransplantation, the hippocampal implant has a fairly mature appearance. Small (CA1) and large (CA3-4) pyramidal neurons are present which often are organized into laminated configurations. A dentate-like subdivision of the implant is observed that contains a well-developed lamina of granule cells with an apical cell-sparse molecular layer and a basal zone containing large (CA4) pyramidal neurons. Thus, the surviving cells within the hippocampal primordium are capable of further cellular reorganization within the transplantation site and continue to develop into a three-dimensional, hippocampal-like structure.
(Supported by NIH grant NS-18126.)

89.6 ENHANCED ACETYLCHOLINESTERASE (AChE) IN THE PERFORANT PATHWAY ZONE AFTER COMBINED LESIONS OF THE SEPTUM AND ENTORHINAL CORTEX. L.L. Chen*, G.W. Van Hoesen, C.L. Barnes* and J.R. West. Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242.

The normal cholinergic innervation of the hippocampal formation is well-known, as is its reinnervation response to ablation of the entorhinal cortex. The system arises from the septum and adjacent basal forebrain. It reaches the hippocampus via the fimbria-fornix, and all components stain for AChE. Little is known about the AChE system of the subicular cortices, whose staining is only altered partially after a septal lesion. We have observed that this system, which presumably arises intrinsic to the hippocampal formation and/or takes non-fornical alternate routes to reach the subicular cortices, will reinnervate the perforant pathway zone of the hippocampus and dentate gyrus when an entorhinal ablation is made along with a septal lesion. In 26 adult male rats bilateral lesions were made in the septum, and the entorhinal cortex was ablated unilaterally in the same setting. After 7-90 days, they were perfused with saline and 4% formalin. The brains were sectioned immediately at 50μ on a freezing microtome and stained for AChE. In 4 rats with combined lesions, perfusion was with sodium sulfide and aldehyde fixatives and the sections were reacted for heavy metals using the Timm's method. After the combined lesion, AChE staining was reduced conspicuously in both hippocampal formations. However, on the side of entorhinal ablation, stainable AChE was present in both the stratum moleculare of the hippocampus and the molecular layer of the dentate gyrus. Its pattern coincided with the known distribution of entorhinal projections to these structures. This staining was continuous with "zone 31" (an AChE positive zone in the molecular layer of the subiculum), that persists after lesions of the septum. In the Timm's stained brains, no alterations were observed in the control hemisphere with the intact entorhinal cortex. However, on the side of the entorhinal ablation dark fibers were observed in the pale staining zones of both the hippocampus and dentate gyrus. As in the AChE stained brains, the pattern covered the terminal area of the perforant pathway. Also, it was continuous with the darkly staining area of the subiculum. These results provide evidence that an intact septohippocampal system is not a prerequisite for reinnervation of the perforant pathway zone by AChE containing axons. Indeed, AChE containing axons associated with the subicular cortices, left intact after a septal lesion, appear to sprout and reinnervate the perforant pathway zone.

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89.7 **TOPOLOGICAL REPRESENTATION IN THE HIPPOCAMPUS.** T.J.Teyler, *Neurobiology Program, NE Ohio College of Medicine, Rootstown, OH 44272.*

O'Keefe and Nadel, among others, suggest that the hippocampus represents either Euclidian space or "cognitive" space. The anatomical organization of the hippocampal formation, coupled with its physiology, appears to permit the registration of information in multi-dimensional space, as will be shown.

The intrinsic architecture of the hippocampal formation features two interleaved sheets of cells, one sheet consisting of the dentate gyrus and the other consisting of the hippocampus. Dendritic processes are oriented normal to the cell sheets. Synapses onto the proximal dendrites of granule cells are from commissural afferents and inhibitory recurrent interneurons. More distally are inputs from entorhinal cortex and medial septum.

At roughly right angles to the longitudinal axis of the hippocampus lies the tri-synaptic circuit. This functional circuit has been used to advantage in the hippocampal slice preparation. This circuit consists of dentate gyrus cells, hippocampal CA3 and CA1 cells. Roughly orthogonal to the tri-synaptic circuit is the hippocampal associational system, which travels longitudinally within and between hippocampi. With the addition of time, the four dimensional nature of the hippocampus is apparent. The four dimensions are: the laminated afferents, the tri-synaptic circuit, the associational system and time.

Within the tri-synaptic path contacts are made *en passage*. This allows a single fiber to synapse with hundreds of neurons along its trajectory. One implication of *en passage* contacts is that a form of neuronal "amplification" can occur. When considered together, these features suggest that appropriately timed beams of neuronal activity can interact on the cell sheets, setting up areas of activation that can be quite small due to lateral inhibitory influences. These patterns of activity can vary in space and time depending upon the nature of the afferent input. These features may permit the hippocampus to represent spatial information.
(Supported by NSF & NIH)

89.8 **ELECTROPHYSIOLOGICAL CHARACTERIZATION OF RAT CLAUSTRAL-ENTORHINAL CONNECTIONS.** C. Hendricks, T.J. Teyler, and Marc Malkoff*. Neurobiology Program, NEOUCOM, Rootstown, OH, 44272

Studies using anterograde and retrograde tracing techniques have recently described the connections between the claustrum and the entorhinal cortex (EC). (Berger, etal, *Neurosci. Abs.*, 7:886, 1981). The purpose of the present experiment was to characterize these pathways using electrophysiolgical methods. The relationship between the claustrum and EC was determined by recording evoked extracellular field potentials and single unit activity. The response profiles evoked by advancing the stimulating electrode through the claustrum to the pyriform cortex while recording in the EC were similar in all animals. The amplitude of a long latency response would rise when the electrode moved into and through the claustrum; no response was evoked as the electrode moved out of the claustrum; and as the electrode approached the pyriform cortex a new shorter latency response was evoked. Because the evoked potentials proved to have consistent response latencies and characteristic shapes, precise placement of the stimulating electrode could be achieved. Single units were recorded in both the claustrum and EC, and the monosynaptic character of their connections was determined by antidromic stimulation techniques. The criteria for single unit antidromic activation were: (1) invariant latency; (2) sharp appearance and stability of threshold; (3) faithful response to high rates of stimulation; (4) one spike per stimulus; and (5) collision of antidromic responses with spontaneous spikes. The response latencies from the claustrum to the EC were long (usually greater than 10ms) especially compared to responses from the pyriform cortex (latencies of 5ms or less). Responses could be elicited in the EC by stimulating many parts of the claustrum, but the strongest responses came from dorsolateral regions. These experiments demonstrate functional monosynaptic connections between the claustrum and EC. Since the claustrum receives input from virtually all of the neocortex and projects to the major afferent to the hippocampus, the EC, it has been hypothesized that the claustrum may serve to relay information between the neocortex and limbic system. Initial investigations show these fibers have the capacity to potentiate.
Supported by NSF.

89.9 LTP AS A CANDIDATE MNEMONIC DEVICE. P. DiScenna and T.J. Teyler. Neurobiology Program, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

The goal of describing the process of information storage in terms of neuroanatomy and neurophysiology has intrigued and challenged scientists for decades. It has been proposed that unspecified changes in synaptic efficacy can encode information over long periods of time (Hebb, 1949).

One potential candidate for such a neural mechanism is long-term potentiation (LTP). LTP is a relatively long-lasting increase in synaptic efficacy observed at certain monosynaptic junctions. It is typically induced by repetitive stimulation of a suprathreshold number of presynaptic fibers.

LTP was first described in the hippocampus (Bliss & Lomo, J.Physiol., 207:61P, 1970). Research on the behavioral effects of bilateral temporal-lobectomy has implicated the hippocampus in disturbances of memory (Scoville & Milner, J.Neurol.Neurosurg.Psychiat., 20:11-21, 1957). Therefore, it is worth considering that LTP may be a candidate for a neural mechanism underlying information storage in the brain.

Since its original description, LTP has been demonstrated at a number of cortical and subcortical loci. Still, the intimate association between LTP and the hippocampus (all three relays of the trisynaptic loop demonstrate LTP) and other closely related limbic structures demands consideration of LTP as a potential mechanism of information storage.

The intent of this consideration is to address the following questions: Are the characteristics of LTP compatible with known aspects of information storage? In particular, we will examine data with regard to: A) the induction, B) time-course, and C) distribution of LTP, D) the effects of pharmacological manipulations and E) the behavioral correlates of LTP.

We propose that such data support a role for LTP in information storage. However, further research focusing on the relationship between LTP and behavior is required before a definitive statement can be made.

Supported by an NSF grant to TJT.

89.10 DEVELOPMENTAL ONSET OF LONG-TERM POTENTIATION IN AREA CA1 OF THE RAT HIPPOCAMPUS. K. M. Harris and T. J. Teyler. Program in Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

The development of hippocampal long-term potentiation (LTP) was studied in area CA1 of rat hippocampal slices. LTP is seen as an enduring increase in the evoked response of hippocampal neurons following tetanic stimulation. Currently, the specific mechanisms of LTP are not fully understood, in part because the adult hippocampus is a very complex structure. It was reasoned that if the developmental onset of LTP production occurred when the hippocampus was relatively immature, in terms of neuronal geometry, that the search for the mechanism(s) of LTP might then be simplified. Therefore, the primary goal of these experiments was to establish when, during development the hippocampus first produces LTP.

In order to compare properties of LTP from animals of different ages, including 1-8, 15, and 60 days following birth, standardized methods for producing maximal levels of LTP in 60 day old animals were applied to all the ages. Tetanic stimulation at 100 Hz was applied for 1 second to fibers of stratum radiatum in area CA1, and postetanus responses from stratum pyramidale were monitored for at least twenty minutes. The magnitude of LTP was calculated at 20 minutes, as the percent increase in the postetanus responses relative to the pretetanus responses. No change was indicated as 0%. LTP was first seen at postnatal day 5, and by postnatal days 7 and 8 the slices from all 8 animals that were tested showed LTP (126±22% increase). Slices from 15 day old animals showed quantitatively more LTP (1823±418% increase) than hippocampal slices from any other age that was tested. For the first 5 minutes following tetanus, posttetanic potentiation (PTP) was equivalent for 15 and 60 day old animals. Starting at 5 minutes after tetanus, responses from 15 day old animals remained elevated for the entire posttetanus monitor (up to 72 minutes in one experiment), while the responses from 60 day old animals declined to lower stable levels of LTP (568±233% increase). Responses of 7 and 8 day old animals showed a similar pattern of maximal potentiation immediately following tetanus followed by a decay to the lower stable levels of LTP.

When a stimulation rate of 1/15 seconds was applied for 100 stimuli, only the 60 day old animals showed potentiated responses, suggesting that the older animals might be sensitive to a wider range of potentiating stimulus frequencies.

An hypothesis is proposed to show how a peak and decline in LTP production across ages might be mediated by the size of an available pool of plastic synapses, which diminishes as more of the synapses become consolidated by the production of LTP. (Supported by NIH 1F31MH0831301 to KMH and NSF 78-23947 to TJT.)

89.11 DIFFERENCES IN PAIRED PULSE POTENTIATION IN HIPPOCAMPAL SLICES FROM SPONTANEOUSLY HYPERTENSIVE AND WISTAR KYOTO RATS. D. Whitehorn and W.C. Low, Dept. of Physiology, Univ. of Vermont College of Medicine, Burlington, VT 05405.

Spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) differ in a number of behavioral and neurochemical as well as cardiovascular parameters. We studied potentiation of CA1 hippocampal pyramidal cell responses to paired electrical stimulation (35msec interval) of radiation fibers in a slice preparation. Simultaneous recordings were made of field EPSPs and population SPIKEs over a range of stimulus intensities. SHR slices exhibited potentiation of SPIKEs and EPSPs. WKY slices had less potentiation of SPIKEs and no potentiation of EPSPs.

Forty slices were obtained from adult animals and equilibrated for 45 minutes in Krebs solution with glucose. Micropipettes were visually placed in stratus radiatum for EPSP and in stratum pyramidal for SPIKE recording. Computer analysis was used to measure spike amplitude at the peak negativity of the initial spike, and EPSP amplitude 1 msec after EPSP onset. Stimulation was applied with a concentric bipolar electrode to stratum radiatum proximal to CA2. Intensity was graded in 10-20 steps until both control (response to first stimulus) and potentiated (response to second of the paired stimuli) responses were maximized. The relationship of SPIKE or EPSP amplitude to intensity displayed a linear range, followed by a region of saturation. The degree of potentiation was quantified by subtracting the control response from the potentiated and dividing by the control.

Both SHR and WKY slices exhibited SPIKE potentiation. In the linear range mean SHR potentiation was 169.5±2.6%, mean WKY potentiation was 91.0±1.5%. In the saturated region mean SHR potentiation was 55.3±1.2%, mean WKY potentiation was 30.6±0.4%. SHR potentiation was significantly greater than WKY in both regions ($p<.005$ and $p<.025$ respectively).

For the EPSP, only SHR slices exhibited a potentiation while the WKYs displayed a depression in response. In the linear range the mean SHR potentiation was 26.2±1.1%, and the mean WKY depression was -12.0±0.9%. In the region of saturation, the mean SHR potentiation was 13.0±1.2% and the mean WKY depression was -14.4±0.8%. SHR EPSP responses were significantly different from WKY responses in both linear and saturated regions ($p<0.005$ and $p<0.05$ respectively).

The failure of WKY slices to exhibit EPSP potentiation and the smaller degree of SPIKE potentiation in the WKY as compared to the SHR may, in part, be responsible for the observed behavioral differences in the two strains of rat. (Supported by HL24110 (DW) and HL 06338-01 (WCL).

90.1 TRACKING ANKLE STIFFNESS DYNAMICS DURING FATIGUING CONTRACTIONS I.W. Hunter, R.E. Kearney and P.L. Weiss. Biomedical Engineering Unit, Faculty of Medicine, McGill University, Montreal, Canada.

The relative contribution of physiological, biomechanical and biochemical changes to muscle fatigue are still neither well understood nor adequately quantified. We have previously developed and demonstrated a combined experimental and analytic technique, based on engineering system identification principles, with which we have been able to quantify changes in the visco-elastic properties of the neuromuscular system controlling the human ankle joint in response to variations in mean muscle force. We now report the results obtained from applying this paradigm to the determination of changes in elastic and viscous terms during sustained, fatiguing contactions of tibialis anterior using human subjects.

Subjects were exposed to stochastic ankle angular position perturbations while maintaining (aided by visual feedback) a constant mean dorsi-flexing torque which was 50% of their maximum voluntary torque. The experimental technique enabled identification of the ankle dynamic stiffness transfer function every 2.5s until subjects could no longer maintain the required torque. Consequently between 30 and 50 transfer functions were determined for each experimental session. After correcting for the dynamics of our apparatus, the elastic and viscous terms were estimated by fitting a second-order transfer function to each successive 2.5s segment of recorded ankle angular position and torque data. The variance accounted for by this dynamic model typically ranged from 90 to 95% and was not correlated with the segment occurrence time. Despite the monotonically increasing tibialis anterior mean rectified EMG which occurred, as expected of a fatiguing muscle, the stiffness transfer functions remained essentially constant throughout a given contraction. The elastic term was invariant although there was a small but we feel functionally insignificant change in the viscous term.

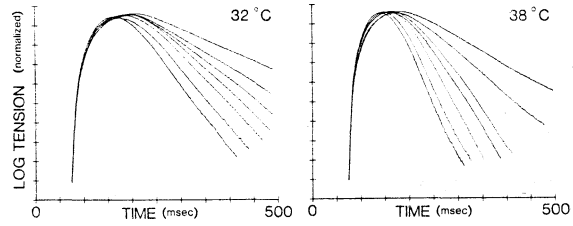
Thus our results show that decrements in human tibialis anterior performance which would commonly be referred to as fatigue, do not arise from changes in the overall muscle-joint biomechanical properties.

Supported by Canadian MRC (R.E.K) and Canadian MDA Postdoctoral Fellowship (I.W.H).

90.2 **THE DEPENDENCE OF TWITCH DURATION ON MUSCLE LENGTH IN CAT SOLEUS.** **R.E. Poppele, P.A. Iaizzo* and B.A. Johnson*. Laboratory of Neurophysiology, University of Minnesota, Minneapolis, MN 55455.**

Duration of isometric twitch tension in soleus muscle depends on muscle length. The half-width of twitch tension increases 22.5% for each 5% increase in length. When the muscle contracts against a load that allows it to shorten as little as 0.3%, the dependence of half-width on muscle length is reduced by 38% (to 14% for a 5% increase in length). Furthermore, the half-width of an isotonic twitch increases only 10% for a 5% increase in the length at which the twitch is evoked. Therefore, the factors that are responsible for the changes in twitch duration do not appear to depend on initial length and they are very sensitive to even small amounts of muscle shortening during a contraction.

Since the time to peak tension varies only slightly with muscle length, the major factor in the increase of twitch duration is the relaxation. Logarithmic plots of twitch tension at different muscle lengths (see below) reveal a nearly exponential fall in tension dominated by a single rate constant. Increases in twitch duration involve both a delay in relaxation from peak tension and decreases in the relaxation rate constant. At the shorter lengths, relaxation rates are nearly identical and relaxation is increasingly delayed with muscle length. Relaxation rate decreases most at the longer muscle lengths. Changes in relaxation rate constant with muscle temperature (28-40° C) are linear on an Arrhenius plot and consistant with an activation energy of 13.2 ±0.3 Kcal/ M, which is the same that has been observed for Ca++ uptake by sarcoplasmic reticulum. Supported by NSF Grant 81-19871.



90.3 FATIGUE-INDUCED CONTRACTILE CHANGES IN MOTOR UNITS OF A FAST-TWITCH MUSCLE. H. P. Clamann, T. M. Beairsto* and L. Dubose*. Dept. of Physiology, Medical Coll. of Virginia, Richmond, Va. 23298

Muscle force is graded by two mechanisms: varying the number of active motor units (recruitment) and varying the discharge rate and hence the force of partially fused tetani (rate coding) of individual units. Rate coding depends strongly on the rise and fall times of twitches, and these speed-related properties can change during motor unit potentiation and fatigue. The present experiments were designed to study changes in the mechanical properties of motor units which occur during isometric fatiguing contractions. Axons of single motor units of cat medial gastrocnemius (MG) were isolated in fine ventral root filaments, and twitch and tetanic characteristics of the muscle units were measured with an isometric force transducer attached to the distal tendon of MG. Each motor unit was classified S, FR, or FF according to the method of Burke, and only units which could be classified unambiguously were studied. A single stimulus was interpolated between successive 40 pps unfused tetani so that changes in twitch properties could be measured during determination of fatigue index. After a recovery period of 30 minutes or more, the unit was driven with 600 msec trains at 3/4 the tetanic fusion frequency, repeated every second. Single stimuli producing twitches were interpolated between these trains. Twitch time to peak, ½-relaxation time and the rise and fall times of tetani were measured.

Units of types FR and FF behaved in a qualitatively similar way. Twitch contraction and ½-ralaxation times increased, as did tetanic rise and fall times. Tetanic fusion frequency fell, often to below 3/4 the initial value with the onset of fatigue. These changes could be produced in 30 seconds or less in some FF units.

Type S units showed no measureable slowing in twitch or tetanic rise or fall times during the two-minute course of intermittent stimulation at 40 pps. Not surprisingly, stimulation at 3/4 the tetanic fusion frequency, generally < 40 pps, produced no slowing either.

These results confirm studies showing a slowing of contraction times in whole, voluntarily contracting muscles(Jones, 1981)† and suggest that the degree of slowing depends on muscle fiber composition. Further, the observation that motor units fuse at lower frequencies when fatigued than when fresh provides a rationale for the observed slowing in EMG frequency during fatiguing contractions as suggested by Bigland-Ritchie (1981).†

(Supported by a grant from the A.D. Williams Foundation)

†In: Human Muscle Fatigue: Physiological Mechanisms; Ciba Foundation Symposium 82; Pitman Medical, London, 1981.

90.4 THIXOTROPIC PROPERTIES OF RELAXED MUSCLES AND JOINTS IN THE RAT. A. W. Wiegner* and R. R. Young. Lab. of Clinical Neurophysiology, Massachusetts General Hospital, Boston, MA 02114.

The viscosity of a thixotropic substance decreases as its shear rate increases -- for example, as a thixotropic liquid is stirred. Walsh (J Physiol 305: 73P, 1980) described thixotropic effects at a number of human joints. That is, when the relaxed joint is subjected to a small sinusoidal torque, the amplitude of the steady-state response is increased up to several fold by a transient larger perturbation. The original state is restored by several seconds of inactivity. Synovial fluid (within joints and tendon sheaths) has been shown to be thixotropic, although this is not considered a significant factor in joint lubrication during movement. The present studies were undertaken to investigate the contributions of muscles to thixotropic effects at a joint and to consider possible physiological consequenses of these effects.

Rats were anesthetized with sodium pentobarbital (60mg/kg) and the lower limb immobilized with two screws through the tibia. Very small sinusoidal torques ($5x10^{-4}$ N-m) were applied to the foot (using a General Scanning G300PD galvanometer with integral position transducer) to rotate it about the ankle. We demonstrated thixotropy at this "intact joint" and also in preparations in which (1) the gastrocnemius-soleus muscle group was replaced by a steel spring, or (2) all tendons were severed at the ankle ("isolated joint"). Furthermore, we observed muscle thixotropy when sinusoidal force was applied directly to (1) the Achilles tendon (detached from the calcaneus) without alterations of the more proximal muscle, its perfusion, or innervation, and (2) freshly excised soleus muscle in a special chamber at 37°C. Thixotropy was absent following the onset of rigor mortis and when fresh muscles were fixed with 10% formalin; thixotropy was never observed in steel springs or rubber bands.

A 1 Hz square wave of torque (1 msec rise-time) was imposed on excised muscles as well as intact and isolated ankle joints. All position responses were characterized by oscillations in velocity and, on occasion, momentary reversals of direction approximately 20 msec following the onset of the torque step. These oscillations, together with the great sensitivity of muscle spindle primary endings to small perturbations, are likely to account for multiple bursts of Ia activity observed with sudden displacements of the human wrist (Hagbarth et al., J Physiol 312: 81, 1981). However, the position response to the first step change in torque was substantially more damped than successive responses. Thus, thixotropic effects, common to both joints and "relaxed" muscles, are of themselves very small, but, through viscous damping, may reduce minute fluctuations in muscle length which might otherwise generate "afferent noise" from spindles.

90.5 TORQUE-VELOCITY AND ARCHITECTURAL RELATIONSHIPS OF KNEE AND ANKLE FLEXORS AND EXTENSORS IN HUMANS. P.L. Powell*, T.L.Wickiewicz*, R.R. Roy, J. Perrine* and V.R. Edgerton (SPON: E. ELDRED). Brain Research Institute and Kinesiology Dept., UCLA, LA, CA 90024.

Isolated skeletal muscle has a predictable force-velocity (F-V) relationship when maximally stimulated. However, in vivo isokinetic (constant speed) testing of knee extensors (KE) shows a lower than expected tension at the high torque-low velocity end of the curve (Perrine, J. and V.R. Edgerton, Med. Sci. Sports 10: 159, 1978). To determine if this phenomenon holds for other functional groups having similar biochemical properties, angle specific F-V curves were generated for knee flexors (KF), plantarflexors (PF), dorsiflexors (DF) and KE at speeds of 0-288 deg/s. Peak torques occurred at slightly greater than 0 deg/s for the KE, KF and PF and at 0 deg/s for DF. For all groups, torques at 0 deg/s were lower than would be predicted based on torques at the higher velocities and on the characteristic in situ F-V relationship. Muscle weights, fiber lengths, fiber angles and sarcomere (sarc.) lengths were determined for prime movers of the knee and ankle on 3 cadaver limbs. Tendon excursions were measured over a 90 degree arc for the knee on 2 limbs. Similar information for the ankle was obtained from Ambagsteer (Acta Orth. Scand. Suppl. 172: 1, 1978). This information allowed us to estimate the maximum intrinsic shortening velocity (Vmax/1000 sarc.), maximum tension (Po) and the tension per cross sectional area (CSA). There was more than a 2-fold range in fiber lengths between muscle groups. Angular Vmax was similar in KF and KE and for PF and DF but these two pairs differed by almost 2-fold. CSA reflects a priority for force production in antigravity muscle groups, particularly for the ankle joint. Considering muscle weight, the soleus and popliteus have a muscle fiber arrangement with the greatest priority for force and the least for displacement. In contrast, the sartorius and gracilis are examples of muscles which maximize displacement and velocity.

	SARC.IN SERIES ($x10^4$)	VMAX (deg/s)	VMAX (mm/s /1000 sarc.)	ΔPO / Δdeg/s	CSA (cm^2)	PEAK TORQUE (kg.m)	EST. P_o (kg)
KF	3.54	680	3.17	.52	42	11.8	92.4
KE	3.09	718	3.06	.15	87	15.0	191.4
DF	2.93	462	1.48	.09	17	1.9	37.4
PF	1.56	392	1.09	.06	137	7.3	301.4

Supported by NIH grant (NS16333)

90.6 MOTOR-UNIT TYPES IN THE CAT FLEXOR CARPI RADIALIS MUSCLE. B. R. Botterman, G. A. Iwamoto and W. J. Gonyea*. Dept. of Cell Biology, The Univ. of Texas Hlth. Sci. Ctr., Dallas, TX 75235

Previous investigators have shown that motor units (MUs) can be divided into three or four types on the basis of the mechanical properties of their muscle units. The classification scheme itself has been successfully applied to several cat hindlimb muscles. Whether it can also be applied to distal forelimb muscles, whose motor tasks in many respects differ from those of hindlimb muscles, is not known. The present experiments were undertaken to determine the applicability of the MU classification scheme of Burke to the forelimb flexor carpi radialis (FCR) muscle, one of two primary wrist flexors.

MU mechanical properties were studied by stimulation of single motor axons, functionally isolated in finely divided ventral root filaments following removal of several segments of the spinal cord. In 7 cats, either anesthesized with α-chloralose (6) or unanesthesized (1) following anemic brain destruction, 53 MUs were studied. The MU population of FCR was readily divided into the 3 main MU types on the basis of contraction time (CT) and resistance to fatigue. There was a clear separation of the MU population on the basis of CT, with fast-twitch MUs having CTs <22 ms and slow-twitch units >26 ms. Mean values (± SD) for selected MU properties of the 3 main MU types are given below.

Motor unit type:	S	FR	FF
Number	23	17	12
CT (ms)	41.7 ± 10.8	15.7 ± 3.7	18.0 ± 3.1
Tetanic tension (g)	4.3 ± 1.9	24.6 ± 17.1	63.1 ± 16.6
Cond. velocity (m/s)	78.4 ± 10.7	100.3 ± 12.0	98.2 ± 8.7

Due to their brief CTs and 1/2 relaxation times, many of the fast-twitch units did not show appreciable increases in force, i.e. above 20% of maximum tetanic tension, until stimulation rates were above 40 pps. Additionally, the fast-twitch MUs were very prone to post-activation potentiation due to sub-tetanic contractions. As a consequence, both FR and FF units demonstrated marked potentiation (2.5X to 20X the initial contraction) during the test for MU fatigue (tetanic stimulation at 40 pps), whereas the type S units showed only slight (1.25X) or no potentiation effects. Thus, it appears that FR and FF units in FCR have the capability to increase force production during high, sustained rates of stimulation. Post-activation potentiation may delay the onset of fatiguing contractions.

In summary, it appears that the classification scheme developed for hindlimb MUs applies also to FCR MUs. Furthermore, the 3 unit types can be identified on the basis of their potentiation properties during fatiguing contractions.

90.7 HISTOCHEMICAL DESCRIPTION OF THE MUSCLES OF MASTICATION OF THE RABBIT. K. Sasamoto*, J.P. Lund, J.-M. Peyronnard* and L. Charron* (SPON: J. Courville). Centre de recherche en sciences neurologiques, Université de Montréal, C.P. 6128, Succ. A, Montréal, Québec, Canada H3C 3J7.

Three species of animals are commonly used in studies of mastication, cat, macaque and rabbit. Histochemical studies of the muscles of mastication of the cat and monkey have shown that there are important differences in the distribution of the three main fiber types, slow (S), fast fatigue-resistant (FR) and fast fatigable (FF) between muscles and, particularly, between the two species. The temporalis, masseter and medial pterygoid muscles of the cat contain a much higher proportion of FF fibers than do those of the monkey (Taylor et al., Exper. Neurol. 38: 99, 1973; Clark and Luschei, Exper. Neurol. 74: 654, 1981). Indeed, the monkey medial pterygoid contains only S fibers.

It is probable that the differences in histochemistry between the species are due to the different way in which the muscles are used to ingest food. Therefore we decided to study the major muscles of mastication of the rabbit, which differ anatomically from those of the other two species and which are used in different ways. The muscles were dissected and the masseter and medial pterygoid muscles were divided into their various layers. Specimens were frozen and complete transverse sections were cut at 12 μm in a cryostat. These were stained for succinic dehydrogenase and myofibrillar adenosine triphosphatase at pH 10.6 and 4.4. Representative areas of each section were photographed. Fibers were classified as S, FF or FR by their staining properties and their mean diameters were estimated using a particle size analyser. All muscles contained a fairly even distribution of the three fiber types. There were minor regional differences within muscles and between the layers of the masseter and medial pterygoid muscles, and to some extent, within the layers themselves. The preliminary data from most muscles are shown below.

Fiber type (%)	S	FR	FF
Masseter - Superficial layer	32%	33%	35%
- 2nd layer	40%	25%	35%
Zygomatico-mandibular	30%	20%	50%
Temporalis	30%	31%	39%
Anterior Temporalis	56%	24%	20%
Retractor	35%	33%	32%
Med. pterygoid - Superficial layer	34%	66%	
- 2nd layer	57%	43%	
Digastric	42%	58%	

The lack of clear histochemical specialization make it unlikely that there are any particular muscles or regions responsible for "vernier" contractions in the rabbit. (Supported by the MRC).

90.8 COMPARTMENTALIZATION OF MOTOR UNITS IN THE MUSCLE BIVENTER CERVICIS OF THE CAT. J. B. Armstrong*, F. J. R. Richmond and P. K. Rose. Dept. of Physiology, Queen's University, Kingston, Ontario Canada K7L 3N6.

Studies of muscle function increasingly recognize that some muscles are compartmentalized into subsections whose motor control may differ. Neck muscles may provide the best model for considerations of compartmentalized muscle function. Anatomical inspection shows that most neck muscles are subdivided into zones by inscriptions of intramuscular tendon. Individual zones appear to be innervated by separate nerve bundles from different spinal segments.

In present studies, 3 methods have been used to provide a more detailed understanding of the fibre organization and innervation of the neck extensor muscle, biventer cervicis. 1) Microdissection techniques were used to describe relations between tendinous inscriptions and extrafusal fibre attachments. 2) Single nerve bundles were stimulated to deplete extrafusal fibres of glycogen. Serial muscle sections were stained with PAS and ATPase methods to define the territory served by each nerve bundle. 3) Motoneurones were stimulated intracellularly to produce glycogen depletion in single motor units. The distribution and histochemical profile of each motor unit were then studied.

Biventer cervicis is anatomically divided into 5 serially linked subsections. The most rostral subsection is composed of fibres which run from the most rostral inscription to the lamboidal crest. More caudal subsections are composed of extrafusal fibres that insert into progressively more caudal tendinous inscriptions. No extrafusal fibres could be demonstrated to penetrate through the inscriptions. Each muscle subsection is innervated by only one nerve bundle which was shown by glycogen depletion to limit its terminal distribution to the muscle fibres of that subsection.

Individual muscle subsections varied in the complexity of their fibre arrangements. Some muscle subsections contained at least 3 different regions of fibres which varied in length and point of origin. Examinations of 14 depleted FF and FR motor units in 2 different subsections showed that a single motor unit occupied only a restricted territory within the subsection, so that all its fibres had a similar length and origin. Such an elaborate pattern of sub-compartmentalization must impose special motor constraints to coordinate the actions of many subsections which are linked to one another both in parallel and tandem.

(Supported by MRC of Canada).

90.9 PROPERTIES OF THE MUSCLES IN THE DIMORPHIC CLAWS OF CALIFORNIAN SNAPPING SHRIMP. P.J. Stephens, J.M. Leferovich*and K. O'Connor* Villanova Univ., Dept. of Biology, Villanova, Pa. 19085.

In alpheid shrimp the first pair of chelipeds are dimorphic and consist of a smaller pincer claw and a larger snapper claw. Removal or denervation of the snapper causes the remaining pincer to enlarge and ultimately transform into a new snapper claw (Mellon and Stephens, Nature 272, 246-248, 1978). We have examined the differences in the properties of the muscles in pairs of pincer and snapper claws to get an indication of the types of changes that take place as a pincer transforms into a new snapper claw.

In Alpheus californiensis each claw has a single opener and a single closer muscle. Sarcomere length measurements made from muscle fibers fixed at resting length revealed smaller values for pincer fibers than for their counterparts in the contralateral snapper claw. Furthermore, in the pincer closer short (2.5uM) sarcomere fibers are confined to the central portion of the muscle, while intermediate length sarcomeres (8.5 to 9uM) are found in fibers located in the ventral and dorsal regions. This regional distribution of different muscle fiber types has been confirmed by examining myofibrillar ATPase activity in frozen transverse sections of pincer claws. Therefore we conclude that a band of fast fibers is located in the center of the pincer closer muscle. The snapper closer muscle, by contrast, is composed of fibers with long sarcomeres and low ATPase activity.

Gel electrophoresis of whole muscle and of myosin extracts revealed differences between pincer and snapper closer muscles.

Our observations indicate that during transformation the fast and intermediate fibers in the pincer closer muscle undergo structural, histochemical and biochemical changes to become slow muscle fibers in the snapper claw.

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90.10 MECHANICAL PROPERTIES OF A SLOW CRUSTACEAN MUSCLE. William D. Chapple. Physiology Section, Biological Sciences Group, University of Connecticut, Storrs, CT. 06268.

Mechanical properties of a slow crustacean muscle, the ventral superficial muscles (VSM) of the hermit crab, Pagurus pollicarus, were studied to obtain the relationship between activation level and 1) force and length, 2) force and speed of shortening, and 3) active stiffness. The VSM was connected to a servo-controlled electromechical transducer and maintained in a constant temperature bath at 12 degrees centigrade in oxygenated saline to which 1 mM glucose was added. When operating within the physiological range, increasing levels of muscle activation resulted in an increase in the slope of the length-tension curve with little shift in peak tension. Force-velocity relations were measured by subjecting the muscle to isovelocity releases and measuring the force at the optimum length. At maximal activation the intrinsic speed of shortening was approximately 15 micrometers per second and the curvature 0.1, consistent with the morphological characterization of the muscle as slow (sarcomere length of 10.8 micrometers and A band length of 5.6 micrometers). At lower levels of activation, the force velocity relationship did not fit the Hill equation, due to partial activation of some sarcomeres. Isovelocity stretches showed an early short range stiffness and a later 'terminal' stiffness, one third that of the short range stiffness. This value is greater than that observed in mammalian muscle. By using a normalizing procedure (Morgan 1976), cross bridge compliance was determined to be similar to that of vertebrate muscle. Non cross bridge compliance was estimated to be five times as great as vertebrate muscle, after correction for a substantial 'passive' parallel elastic component. The response of this isolated muscle to stretch thus resembles the responses of reflex compensated cat muscle to stretch (Nichols and Houk 1976). These features can be viewed as particularly appropriate in adapting a muscle to postural tasks.

90.11 MUSCULAR DYSTROPHY OF THE CHICKEN: EFFECTS OF CORTICOSTERONE AND ISOPROTERENOL. R. K. Entrikin, G. T. Patterson, and B. W. Wilson. University of California, Davis, CA 95616.

There are no effective therapies for most forms of muscular dystrophy in man, including the usually fatal Duchenne type. Since the causes are also unknown, there are literally thousands of possible therapies to consider. Unfortunately there are many limitations to clinical trials and use of human dystrophic cells in culture. One alternative is to select compounds on the basis of pre-clinical trials in experimental animal "models." As part of that effort we have developed a multi-phase system to evaluate 200 or more drugs annually in genetically dystrophic chickens. Phase-I is designed to rapidly identify compounds that alleviate impaired righting ability of dystrophic chicks. Highly effective compounds are examined in longer-term, Phase-II evaluations for effects on other dystrophic features such as increased plasma creatine kinase (CK) activity and muscle histology. Both Phases employ a strict "blind" to eliminate bias.

Every other week 120 newly-hatched line 413 dystrophic chicks are placed six per cage and injected i.p., (once-daily on days 2-4 ex ovo; twice-daily thereafter) with either a standard diluent (controls) or specific test compound. On days 15 and 22 ex ovo righting ability is determined by the exhaustion score method (ES), the consecutive number of times a chick can rise from the supine in immediate succession during a single trial.

Of over 100 compounds examined to date only corticosterone-21-acetate (C-21-A) and isoproterenol (ISO) consistently produced higher mean ESs than the positive control, methysergide maleate (MES). C-21-A has been studied in 30 trials; ISO in 12 trials. In Phase-II the highest doses of C-21-A (18 mg/Kg) reduced plasma CK activity by as much as 90% and reduced the incidence of abnormally large, rounded fibers in the dystrophic pectoralis major muscle. In its first Phase-II evaluation ISO reduced plasma CK activity by 32% at 0.7 mg/Kg, and by 80% at 3.5 mg/Kg. Below are data summaries from single trials with normal control (412), dystrophic control (413), C-21-A-treated dystrophic (9 mg/Kg), ISO-treated dystrophic (3.5 mg/Kg), and MES-treated dystrophic (2 to 12 mg/Kg) groups. (Means ± SEM; "n" in parentheses).

Group		15d ES	22d ES	36d CK (mU/ml)
412	(18)	16.8 ± 4.4	17.5 ± 3.8	178 ± 51
413	(6)	4.0 ± 2.1	1.6 ± 1.6	1997 ± 534
MES	(6)	5.3 ± 1.6	5.2 ± 1.8	---
C-21-A	(6)	8.8 ± 1.7	14.7 ± 2.9	544 ± 84
ISO	(6)	11.3 ± 2.4	11.3 ± 3.0	416 ± 165

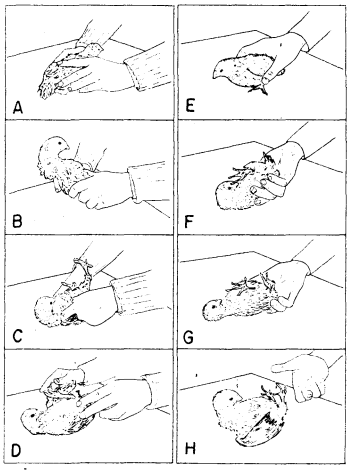
(Supported by the Muscular Dystrophy Association Task Force On Drug Development).

90.12 DISTRIBUTION OF ACETYLCHOLINESTERASE MOLECULAR FORMS IN ORGANS OF DYSTROPHIC MICE. K.A. Skau. Dept. Biochem. Pharmacol. and Toxicol., University of Utah, Salt Lake City, Utah 84112.

An aberration of the membrane bound (G_4) form of Acetylcholinesterase (AChE) has been reported in hemidiaphragm (HD) and extensor digitorum longus (EDL) of the genetically dystrophic mouse (ReJ/129). As the G_4 form is widely distributed in mammalian tissues, the present experiments were designed to examine levels of AChE and the distribution of the forms of this enzyme in various organs of dystrophic mice. Organs from ReJ/129 (dy/dy) dystrophic mice and clinically normal (+/?) littermates were homogenized in tris-buffered medium containing 1% Triton x-100 and 1M NaCl to solubilize AChE. Low speed supernatants from these homogenates were centrifuged on 5-20% linear sucrose density gradients to resolve the AChE molecular forms. No quantitative or qualitative differences were observed in AChE from heart, thymus, salivary glands, lung, liver, spleen, pancreas or gut tissue. AChE activity in serum and erythrocytes was also normal. Absolute levels and the distribution of the molecular forms in 24 hr. ligated sciatic nerves or contralateral, unligated nerves were similar for dystrophic and control mice. Soleus muscles from dystrophic mice were 50% smaller than from clinically normal mice (4.2±0.67 mg vs. 8.4±0.49 mg; $p < 0.001$); however, total AChE was not different (1.97±0.43 mU in dy/dy vs. 1.86±0.33 mU in +/?). Neither dystrophic nor clinically normal soleus muscle had a significant G_4-AChE peak, unlike HD and EDL muscles which have prominent G_4 peaks from control but not dystrophic mice. As with HD and EDL, soleus muscles exhibited an increased G_1-AChE peak. When HD were dissected into endplate rich (EP+) and endplate poor (EP-) regions, the dystrophic EP+ tissues showed normal levels of AChE in the G_4 region but the EP- portion was devoid of G_4-AChE indicating that the previously reported high activity (but lack of a true G_4 peak) is due to intramuscular nerves in the EP+ region. The distribution of the AChE forms of dystrophic mice, i.e. little alteration in A_{12}-AChE, absence of G_4-AChE and enrichment of G_1 enzymes, resembles the distribution of all of the offspring of dy/+ parents during the juvenile stage. These results suggest that the AChE abnormality is not found in visceral organs but is restricted to skeletal muscle and that this abnormality may be a failure of the muscle to mature biochemically. (Supported by a grant from the Muscular Dystrophy Association).

90.13 EVALUATION OF FUNCTIONAL DISABILITY IN GENETICALLY DYSTROPHIC CHICKENS, M. S. Hudecki and R. K. Entrikin, Div. of Cell and Molecular Biology, State Univ. of New York, Buffalo, NY 14260; Dept. of Pharmacology, Univ. of Calif., Davis, CA 95616.

A commonly evaluated functional abnormality in genetically dystrophic chickens is their inability to rise from the supine. This disability is one criterion used to assess drug effects. The aim of this study was to compare methods for evaluating righting ability at State Univ. of NY at Buffalo (SUNY/B) and Univ. of California, Davis (UCD). The protocol involved trials in which identical day 23 ex ovo Line 413 dystrophic chickens were each assessed for righting ability using the SUNY/B Flip Number test (FN - no. of successful righting attempts in 5 opportunities) and the UCD Exhaustion Score test (ES - consecutive no. of successful righting attempts). The two methods are illustrated. The SUNY/B method involves 2-hand positioning, head towards tester (A), rotation in a beak-up pitch motion into supine (B, C), and manually-restrained rest period prior to lateral 2-hand release (D). The UCD method involves single hand positioning, head away from tester (E), rotation in an external roll motion into supine (F), and immediate caudal release of hand (G, H). The SUNY/B method consistently yielded higher FN and ES values than those obtained by the UCD procedure. Several differences exist between the two testing techniques (e.g., temporary restraint prior to release vs. immediate release). Both methods yield highly reproducible results. We conclude that apparent differences in righting ability found at the two institutions are due to the specific testing methods and not to an actual difference in the rate of progression of the chicken myopathy. (Support of MDA, and help of C. M. Pollina and J. Stamos is acknowledged.)



90.14 CHARACTERIZATION OF A HUMAN MUSCLE CELL LINE DERIVED FROM A RHABDOID WILMS TUMOR. K.C.Leskawa*, D.A.Sens*, A.J.Garvin*, E.L.Hogan, M.A.Sens*. Departments of Neurology and Pathology, Medical University of South Carolina, Charleston, SC 29425.

Attempts to cultivate both Wilms tumor and primitive human muscle beyond organ culture or primary culture have been unsuccessful due to fibroblastic proliferation and overgrowth. Recently, a case of Wilms tumor with focal areas of rhabdoid differentiation was studied and successfully established in tissue culture. The explant technique utilized standard tissue culture media (Dulbecco's modified Eagles' media), supplemented with either fetal calf serum or a mixture of insulin, transferrin, selenium, hydrocortisone, T3 and prostaglandin E1. In all cultures grown in the presence of serum there was considerable fibroblastic contamination. However, on untreated plastic in serum-free media, myoblastic cells proliferated. Beginning at four days in culture, multinucleated tubular structures were formed by fusion of myoblasts. This cell line has been subcultivated through passage nine, with repeated formation of multinucleated contractile elements. Electron microscopy reveals myotubes with readily visible A, I and Z bands, as well as thick and thin filaments. Morphometric analysis of electron micrographs suggests that all cells are of myoid origin with no contamination by fibroblastic or epithelial elements. Collagen and gelatin substratums enhance myoblast differentiation and myotube formation in vitro, whereas poly-L-lysine was much less effective. Cells grown on fibronectin-coated dishes exhibit altered morphology, i.e. loss of normal spindle shape and increased extensions. The effects of various media constituents (e.g. divalent cations, metabolic inhibitors and exogenous enzymes) on the process of myoblast fusion leading to myotube formation will also be presented. It is believed that this cell line, which can be grown and subcultured in serum-free media, represents primitive muscle tissue which, upon repeated passage, continues to form multinucleated myotubes during growth and may therefore serve as a useful model system for myogenesis and other in vitro studies of human muscle.

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90.15 SEXUALLY DIMORPHIC SKELETAL MUSCLE RESPONSE TO GLYCOLYTIC BLOCKADE F.C. Garb*, J.W. Gerst* and R.A. Brumback. Zool. Dept., North Dakota St. Univ. and Neurol. Ser. V.A. Med. Ctr. Fargo, ND 58102.

Among reported cases of human myophosphorylase deficiency (McArdle's Disease) there is a prevalence in males of approximately 4:1 (DiMauro and Eastwood, Adv. Neurol. 17;123, 1977). An animal model (Brumback, Susag and Gerst; Am. J. Path. 101;241, 1980) using sodium iodoacetate (IOA) to selectively inhibit the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase was used to determine if sexual dimorphism exists in the response of rat skeletal muscle to ischemic exercise following glycolytic blockade and, if so, to determine the influence of estradiol on this difference.

IOA was delivered to anesthetized male and female Wistar/Furth rats by intraaortic injection at a dose of 25 mg/kg. One hour after injection the hindlimb muscles were exercised by stimulating indirectly through the sciatic nerve using supramaximal 0.075 msec pulses at 5 Hz. The abdominal aorta was occluded producing ischemia in the hindlimb during exercise. Three pre- and post-exercise aspects of the hindlimb were compared to assess the degree of induced contracture: 1) the angle through which the foot could be moved 2) the distance between the first and fifth digit; and 3) the distance between the fourth and fifth digit.

Treatment groups included intact males and females and ovariectomized females with and without 17β-estradiol replacement therapy. The influence of the stereoisomer, 17α-estradiol was examined in female rats. Analysis of variance revealed significant between-group differences in the means of contracture indicies (Table I). There is sexual dimorphism in the response; male rats underwent a more intense degree on contracture. Ovariectomy caused an increased intensity of contracture in females. 17β-estradiol prevented this increase; 17α-estradiol did not.

Table I. PERCENT OF CHANGE IN RAT HINDLIMB DUE TO EXERCISE-INDUCED CONTRACTURE FOLLOWING IOA INJECTION

Condition	Angle of Movement	Distance: Digit 1-5	Distance: Digit 4-5
Intact Males (11)	-86 ± 2*	60 ± 8*	240 ± 40*
Intact Females (13)	-67 ± 6	29 ± 6	117 ± 26
Ovx+17b-E (20)	-73 ± 2	26 ± 4	106 ± 21
Ovx (11)	-84 ± 2*	52 ± 6*	205 ± 38*
Ovx+17a-E (3)	-82 ± 1*	52 ± 10*	260 ± 105*

*different from intact females

The effect of estradiol receptor blocking agent (CI-628) on the sparing influence of 17β-estradiol in females and the effects of 17β-estradiol on intact and orchiectomized males is being evaluated.

This work was supported in part by the Veteran's Administration.

90.16 GLUCOCORTICOID TREATMENT DOES NOT ALTER MUSCLE MEMBRANE EXCITABILITY. R. L. Ruff, D. Martyn*, W. Stühmer*, W. Almers*, and A. M. Gordon. Department of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

Excess of endogenous or exogenous glucocorticoids leads to muscle atrophy and weakness with preferential involvement of proximal muscles and Type II muscle fibers. Greuner & Stern (Arch. Neurol. 26:181-185, 1972) suggested that steroids might act by depolarizing Type II muscle fibers causing a decrease in action potential amplitude, thereby reducing force generation. According to this hypothesis, the muscles should become weak before they atrophy. To test this hypothesis, the temporal pattern of muscle wasting, in vitro twitch and tetanic tensions; an in vitro and in vivo resting membrane potentials, excitability, and passive membrane properties were studied in the soleus (SOL), extensor digitorum longus (EDL), semimembranous (SM), and omohyoid (OMO) muscles of control and steroid intramuscular dexamethasone or cortisone acetate treated rats. One week of steroid treatment produced significant atrophy in the EDL, SM, and OMO. The soleus did not show weight loss until three weeks of treatment. After four weeks of steroid treatment, the order of severity of muscle atrophy was OMO$\geq$SM>EDL>>SOL. The decrease in twitch or tetanic tension always followed muscle atrophy, which argues against the tested hypothesis. In all the muscles, the twitch or tetanic force/g of muscle increased with steroid treatment. The twitch/tetanus ratio increased for the EDL and SOL, decreased in the SM and was not changed in the OMO. In vitro recorded resting membrane potentials were depolarized only in the EDL. This depolarization was seen after one day of dexamethasone treatment or one week of cortisone acetate treatment. This depolarization was not seen in vivo. Steroid treatment did not alter the in vivo measured action potential threshold passive membrane properties. or $[Na]_{i,o}$ and $[K]_{i,o}$. Supported by NIH NS00498, NS16696, NS08384, AM 17803.

90.17 BIOCHEMICAL CHARACTERIZATION OF INHIBITION OF SARCOPLASMIC RETICULUM (Ca^{2+} + Mg^{2+}) ATPase BY PUMILIOTOXIN-B. P.M. Sokolove,* E.X. Albuquerque, J.W. Daly & F.C. Kauffman. Dept. Pharm. & Exp. Therap., U. of MD, Sch. of Med., Baltimore, MD 21201 & Lab. of Bioorganic Chemistry, NIH, Bethesda, MD 20205.

Pumiliotoxin-B (PTX-B), an indolizidine alkaloid isolated from the neotropical frog Dendrobates pumilio, has been reported to be a potent and specific inhibitor of the (Ca^{2+} Mg^{2+}) ATPase of skeletal muscle sarcoplasmic reticulum (Tamburini, et al., 1981, J. Neurochem. 37:551). Characterization of the mechanism of inhibition was undertaken because this agent has been shown to alter Ca^{2+}-dependent physiological responses in nerve and muscle. Moreover, preliminary findings indicate that PTX-B inhibits the SR ATPase of normal, but not of dystrophic, muscle (Albuquerque et al., 1982, Disorders of the Motor Unit, (D.L. Schotland, ed.) J. Wiley and Sons, Page 611).

Using SR vesicles prepared from rat skeletal muscle, we have obtained the following results: (1) PTX-B inhibition of SR ATPase activity is dependent on the ratio of toxin to protein, with 50% inhibition occuring at ca. 650 PTX-B/ATPase. A protein concentration of 2 µg/ml was used routinely in assays. (2) Inhibition is enhanced significantly by an increase in temperature from 25 to 37°C; PTX-B decreases the absolute value of the slope of the Arrhenius plot for ATPase activity. (3) PTX-B inhibition is antagonized by an increase in either ATP or Ca^{2+} concentration. Increasing concentrations of ATP enhance catalytic activity and cause a commensurate loss of sensitivity to the toxin. Thus, interaction of ATP with the catalytic site alters the sensitivity to PTX-B. On the other hand, ATPase activity is stimulated by markedly lower levels of Ca^{2+} than are required to reverse PTX-B inhibition. It therefore appears that Ca^{2+} effects on PTX-B inhibition are not mediated by the high affinity Ca^{2+} binding site(s) on the enzyme. Inhibition with respect to ATP is mixed linear noncompetitive with Ki = 15 µM at 37°C and 1.25 µM free Ca^{2+}. The Hill coefficient for ATP is unaltered by PTX-B, suggesting that PTX-B does not alter the interaction between catalytic and proposed regulatory sites for ATP.

The data indicate that PTX-B does not directly compete for either the Ca^{2+} or ATP site(s) on the ATPase, however, PTX-B markedly decreases the affinity of the enzyme for ATP. The lipophilic nature of PTX-B, the dependence of inhibition on protein concentration, and the reduction of inhibition by high Ca^{2+} concentrations suggest that the action of the toxin may be related to alterations in the membrane environment of the ATPase. (Supported in part by NIH Grant NS-12063 and USARMDC Grant NS-14728.)

91.1 LOCALIZATION OF ^{125}I-α-BUNGAROTOXIN BINDING IN CHICK CILIARY GANGLIA. R.H. Loring and R.E. Zigmond, Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

α-Bungarotoxin (αBgT), a neurotoxin derived from the venom of Bungarus multicinctus, blocks neuromuscular transmission by binding to acetylcholine receptors at the neuromuscular junction. α-BgT also binds to high affinity sites in the chick ciliary ganglion and this binding can be inhibited by carbachol and d-tubocurarine. However, since αBgT does not block nicotinic transmission in these ganglia the physiological function of these binding sites is unclear. In order to further characterize these sites we have determined their cellular and subcellular distribution in the ciliary ganglion using light and EM autoradiography. Ciliary ganglia from 18-day chick embryos were dissected, incubated in 20 nM ^{125}I-αBgT for four hours at 37°C, washed for 10 min in Eagle's minimum essential medium, fixed in 2% gluteraldehyde-2% paraformaldehyde and then counted in a γ-counter. Preincubation for 1 hr in either 100 μM d-tubocurarine or 1 μM αBgT decreased ^{125}I-αBgT binding in whole ganglia by 85% and 83% respectively. The ganglia were then processed for light and electron microscopic autoradiography. For the initial analysis at the electron microscopic level, grains were classified as lying over "synaptic contact zone" (within 1.2 μm of a contact between pre- and postsynaptic elements), "neuronal rim" (within 1.2 μm of the neuronal plasma membrane but not within "synaptic contact zone") neurcnal cytoplasm, non-neuronal cytoplasm, myelin, collagen, open space or axoplasm. More than 90% of the specific ^{125}I-αBgT label was found to be associated with neurons and was divided between "synaptic contact zone" (∿ 65%), "neuronal rim" (∿ 10%), and neuronal cytoplasm (∿ 20%). The "synaptic contact zone" of large neurons could be further subdivided into "simple" contact regions (in which there is a close apposition between the plasma membranes of the pre- and postsynaptic neurons) and "complex" contact regions (in which this apposition is interrupted by processes originating from the pre- and postsynaptic neurons). Most of the ^{125}I-αBgT label (> 80% of the grains) was restricted to the "complex" contact regions which may in part reflect the greater membrane area in these regions. Few grains (< 5%) were associated with membrane densities in "synaptic contact zones." Cytoplasmic ^{125}I-αBgT binding was most prominent in small neurons found near the periphery of the ganglion. Grains overlying neuronal cytoplasm were often associated with multivesicular bodies and lysosomes. These results indicate that 1) ^{125}I-αBgT binding in chick ciliary ganglia is specifically localized to neurons, 2) the major portion of this binding is associated with "synaptic contact zones," and 3) a fraction of bound αBgT is internalized. Supported by USPHS grants NS12651, NS07009 and MH 00162.

91.2 NICOTINIC CHOLINERGIC RECEPTORS LABELED BY ^{3}H-ACETYCHOLINE IN RAT BRAIN: IN VITRO AND IN VIVO MODIFICATION. Rochelle D. Schwartz* and Kenneth J. Kellar. Dept. Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington D.C. 20007

We have recently identified a ^{3}H-acetylcholine (^{3}H-Ach) binding site with characteristics of a nicotinic cholinergic receptor in rat brain (Mol. Pharmacol., July, 1982). In the present study we have examined the effects of in vitro and in vivo treatments on the brain binding site chraracteristics. Preincubation of cerebral cortical or striatal membranes with the sulfhydryl reducing reagent dithiothreitol (DTT, 1 mM) decreases the number of ^{3}H-Ach binding sites (B_{max}) by 75% but does not alter the affinity (K_D) of binding. Following preincubation with DTT, reoxidation of the membrane sulfhydryl groups with dithiobis-nitrobenzoic acid (DTNB, 1 mM) effectively reverses the decrease in ^{3}H-Ach binding sites. Alkylation of the ^{3}H-Ach binding sites with 1 mM p-chloromercurobenzoate following reduction with DTT prevents the restoration of binding sites by DTNB. These effects of DTT on ^{3}H-Ach binding site characteristics are different from those produced on ^{125}I-α-bungarotoxin binding and suggest that the two ligands do not label the same site.

To determine whether the ^{3}H-Ach binding site can be regulated by agonists, cortical membranes were preincubated with various nicotinic cholinergic agonists and then washed thoroughly to remove the agonists prior to the binding assay. Preincubation with acetylcholine (10 μM), nicotine (10 μM) or cytisine (1 μM) decreases binding of ^{3}H-Ach by 30-50%, indicating that the binding site can be desensitized by agonists in vitro.

Chronic treatment of rats with nicotine (1 mg/kg, s.c., twice a day for 10 days) significantly increases the number of binding sites (15-35%) in several brain areas while the affinity of the sites is unaltered. In contrast, chronic treatment with the cholinesterase inhibitor, diisopropylfluorophosphate, results in a decrease in the number of binding sites in brain. Thus, ^{3}H-Ach binding sites in rat brain can be altered by modification of critical disulfide bonds and the sites can be regulated by agonists both in vitro and in vivo. These changes may relate to regulation of the receptor binding site and to its function in central nicotinic cholinergic transmission.

91.3 RADIOLABELLED SNAKE α-NEUROTOXINS AS PROBES FOR THE NEURONAL NICOTINIC RECEPTOR IN THE CHICK CILIARY GANGLION. V.A. Chiappinelli. Dept. of Pharmacology, St. Louis Univ. Sch. of Med., St. Louis MO 63104.

Previous work (1,2,3) has demonstrated that several, but not all, α-neurotoxins isolated from Bungarus multicinctus venom block transmission in the chick ciliary ganglion in a manner consistent with their binding to the neuronal nicotinic receptor. In addition, α-neurotoxins which do not block transmission in this ganglion (notably α-bungarotoxin (ABTX)) also bind to a site in the ganglion which is pharmacologically nicotinic in nature. To determine whether these two binding sites are identical, radiolabelled toxins were prepared which were potent blockers of ciliary ganglion transmission. The results obtained in an initial study of a radiolabelled active toxin (4) indicated that the physiologically active α-neurotoxin bound to two sites: the ABTX site and an additional site, which was not seen by ABTX. This additional site did not appear to be nicotinic in nature as 100 μM d-tubocurarine or 5 mM carbachol did not prevent the α-neurotoxin from binding to this site.

Following this initial study, a number of questions arose which led to the purification and radiolabelling of another α-neurotoxin which blocked transmission in the chick ciliary ganglion. This toxin was purified from venom by cation exchange chromatography, blocked transmission through the ciliary ganglion at a low dose (100 nM) and could be distinguished from ABTX and a number of additional α-neurotoxins on the basis of its behavior on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing in polyacrylamide gel (IEF) and carboxymethylcellulose liquid chromatography. The material was radiolabelled to a high specific activity using the chloramine T method and carrier-free Na ^{125}I. Carboxymethylcellulose gradient purification of the iodination products yielded two radioactive toxin peaks. One of the peaks appeared identical with the ganglion blocking material on the basis of SDS-PAGE and IEF. A parallel iodination was done with non-radioactive Na I, and this iodinated material was tested for blocking activity on the ganglion and proved to be active at 100 nM. The binding of the radiolabelled material was explored in three tissues: the chick ciliary ganglion, the striated iris muscle of the chick, and Torpedo electric organ membranes. In all three tissues studied, this toxin bound to a single site which was fully protected by pre-exposure to ABTX. (Supported in part by NIH Grant NS 17574 and a PMA Foundation Research Starter Grant.)

1. Chiappinelli and Zigmond (1978) Proc. Natl. Acad. Sci. USA, 75, 2999.
2. Ravdin and Berg (1979) Proc. Natl. Acad. Sci. USA, 76, 2072.
3. Chiappinelli, Cohen and Zigmond (1981) Brain Res., 211, 107.
4. Chiappinelli, Loring, Cohen and Zigmond (1982) (In Preparation).

91.4 LIPID-DEPENDENT RECOVERY OF HIGH AFFINITY BINDING OF α-BUNGAROTOXIN TO THE PURIFIED α SUBUNIT FROM TORPEDO ACETYLCHOLINE RECEPTOR. Socrates Tzartos and Jean-Pierre Changeux. Neurobiologie Moléculaire, Institut Pasteur, 75724 Paris 15, France.

Acetylcholine receptor (AChR) from fish electric organ has the subunit structure $\alpha_2\beta\gamma\delta$. The α subunit carries at least part of the ACh and α-toxin binding sites. The purified α subunit has been shown to exhibit a low affinity for α-bungarotoxin (K_D 0.1-0.2μM) (Haggerty and Froehner, Biochemistry 256, 8294). We describe here conditions for the recovery of high affinity α-bungarotoxin binding to the isolated α subunit (K_D ~ 0.5 nM) and some characteristic features of this binding.

We purified subunits from Torpedo marmorata AChR by SDS-acrylamide gel electrophoresis after dissociation of the membrane-bound AChR with 2% SDS. The purified subunits were diluted with a buffer containing 0.5% cholate and 1% lipids (asolectin) to a final subunit concentration of ~ 1 nM. SDS was eliminated by an anion exchange resin. The α subunit preparation bound ^{125}I-α-bungarotoxin with K_D ~ 0.5 nM and a half time for dissociation of ~ 3h. The β, γ or δ subunits showed no significant α-bungarotoxin binding. ^{125}I-α-bungarotoxin binding could be inhibited by unlabeled α-bungarotoxin (added before or after the labeled toxin). On the contrary, carbamylcholine (≤ 25 mM), decamethonium (≤ 25 mM), tubocurabine (≤ 1 mM) and the short-chain Erabutoxin b (≤ 10 μM) did not reduce the rate of ^{125}I-α-bungarotoxin binding to the α subunit. We are currently studying the binding of conformation-dependent anti-AChR monoclonal antibodies to the α subunit-toxin complex.

High affinity α-bungarotoxin binding required continuous presence of high concentrations of lipids, even after the toxin had already bound to the subunit. KCl or NaCl (20 mM) and EGTA (1 mM) enhanced α-bungarotoxin binding. Although the subunits could be kept for several days in SDS (prior to dilution in cholate + lipids), even short incubation (1-3 h) in 0.5% cholate, in the absence of lipids, dramatically decreased subsequent recovery of α-bungarotoxin binding. The recovery of α-bungarotoxin binding also depended upon the dilution of the subunits in cholate+lipids prior to SDS elimination. Sucrose gradient centrifugation and gel filtration experiments performed in the presence of cholate + lipids suggest the formation of a high molecular weight complex, but the contribution of the detergent and lipids to this complex is not yet known.

These results show that the α subunit can regain a conformation that is sufficiently close to the native one so that high affinity binding of α-bungarotoxin can occur; however in this conformation, competition for the α-bungarotoxin binding site by other ligands is not recovered.

91.5 CHARACTERIZATION OF THE 43,000 DALTON PROTEINS ISOLATED FROM ACETYLCHOLINE RECEPTOR-RICH MEMBRANES. S. Porter* and S. C. Froehner. Dept. of Biochemistry, Dartmouth Medical School, Hanover, NH 03755.

Highly purified postsynaptic membranes from Torpedo electric organ contain, in addition to the subunits of the acetylcholine receptor, a 43,000 molecular weight peripheral membrane protein (43K protein). Treatment of membranes at pH 11 or with low concentrations of lithium diiodosalicylate (LIS) remove the 43K protein from the membranes without detectable effects on receptor function. We have previously shown by immunofluorescence with rabbit antiserum directed against an alkaline extract that the 43K protein is restricted to the innervated face of Torpedo electrocytes and that an immunologically similar component is highly-concentrated on the cytoplasmic face of the postsynaptic membrane of the rat neuromuscular junction (Froehner et al, PNAS 78:5230, 1981). It has recently been shown that an alkaline extract of Torpedo membranes prepared by an affinity partition method is composed of three proteins of approximately 43,000 mol.wt. differing in isoelectric point (Gysin et al, J. Biol. Chem. 256:11 373, 1981). As a first step in biochemical characterization of this synaptic component, we were interested to ascertain if the 43K protein(s) in membranes that we prepare by a more conventional method has this heterogeneity. Furthermore, it was important to determine which 43K protein(s) is recognized by the antiserum used in our immunofluorescence studies. Torpedo postsynaptic membranes were prepared by a modification of the method of Elliott et al (Biochem. J. 185:667, 1980) and LIS extracts were analyzed by two-dimensional gels (isoelectric focusing and SDS gel electrophoresis). Several major 43,000 mw components of pI 7.0 - 8.0 and a minor component of pI 5.6 were found. In addition, a very small amount of a 40-41,000 dalton protein (pI 7.0 - 7.5) was usually detected. The most acidic protein comigrated on a 2D gel with chicken muscle actin. Furthermore, when the spot was cut out of the gel and analyzed on a one-dimensional peptide map, it gave a pattern which was identical to that of actin. The three major 43,000 dalton proteins of pI 7.0 - 8.0 yield peptide map patterns which are indistinguishable from one another but which are very different from that of actin. The basis for the charge heterogeneity of the 43K protein is as yet unknown. An immunoblot of a 2D gel was performed to determine the reactivity of the antiserum with the proteins resolved by this technique. The results show that, of the LIS-extractable proteins in the range of 40-50,000 mol.wt., only the 43,000 dalton protein of pI 7.0 - 8.0 reacted with the antiserum. There was no reactivity with actin or the 40-41,000 dalton protein. This work was supported by NIH grant NS 14871.

91.6 BINDING OF PHENCYCLIDINE TO THE TORPEDO MARMORATA ACETYLCHOLINE RECEPTOR: EFFECTS OF DETERGENT SOLUBILIZATION AND CALCIUM. R.E. Oswald. Dept. of Pharmacology, N.Y.S. Coll. Vet. Med., Cornell Univ., Ithaca, NY 14853.

Phencyclidine (PCP) binds to the nicotinic acetylcholine receptor (nAChR) and inhibits ion flux at a site distinct from the acetylcholine binding site. The nature of this binding site is still unresolved; however, the voltage-dependence of the inhibition of ion flux suggests that PCP may sterically block the ion channel (Albuquerque et al., (1980) PNAS 77, 1224). Equilibrium binding experiments with [^{3}H]PCP indicate that agonists (acetylcholine, carbamylcholine) increase the affinity of the nAChR for [^{3}H]PCP (K_D = 2.5 μM without agonists; K_D = 0.25 μM with agonists). The order of addition of agonists and [^{3}H]PCP determines the kinetic but not the equilibrium properties of the binding. If [^{3}H]PCP is added following desensitization with agonist (closed ion channels--"prior addition"), the association rate is 10^3 to 10^4 fold slower than that expected for a diffusion controlled reaction. If [^{3}H]PCP and agonist are added simultaneously (ion channels open transiently in the presence of PCP--"simultaneous addition"), the association rate connot be resolved by manual filtration techniques (< 2 sec.) and is apparently diffusion controlled. The dissociation rate is also increased transiently by the simultaneous addition of agonist and [^{3}H]PCP. One possible interpretation of these data is that the binding site is in the ion channel and that when the channel is closed (under desensitizing conditions), a significant diffusion barrier limits the association and dissociation rates. Opening the channel may remove this diffusion barrier and increase both association and dissociation rates.

Solubilization of the nAChR in Na cholate followed by exchange of cholate for Tween 80 yields a soluble receptor molecule capable of conformational changes in response to agonist binding (Heidmann, Cuisinier & Changeux (1981) C.R. Acad. Sci. 292D, 13). Binding of [^{3}H]PCP to the Tween 80 soluble nAChR can be measured by a filtration assay using positively-charged DE81 filters to retain the negatively-charged nAChR. The rapid unresolved binding event observed with the "simultaneous addition" protocol remains in the soluble state. Using the "prior addition" protocol (desensitizing conditions), both the association and dissociation rates are approximately two fold greater in the soluble state than in the membrane-bound state. In both the Tween 80 soluble and the membrane-bound nAChR, calcium decreases the association rate and increases the dissociation rate resulting in a two fold increase in the equilibrium dissociation constant. This effect of calcium is the only observed in the presence of agonists. The effect of detergent solubilization and calcium will be discussed in terms of the known conformational transitions of the nAChR.

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91.7 MONOCLONAL ANTIBODIES TO NICOTINIC ACETYLCHOLINE RECEPTOR CHARACTERIZED BY ELECTRO-TRANSFER TECHNIQUES. Edward Hawrot, Jonathan M. Gershoni*, Thomas G. Burrage, Gregory S. Paladino*, Thomas L. Lentz and Linda L.Y. Chun*. Dept. of Pharmacology and Section of Cell Biology, Yale Univ. Sch. Med., New Haven, CT 06510 and Dept. of Neurology, Mass. General Hospital, Boston, MA 02114.

Mice immunized with detergent-extracted, affinity-purified Torpedo acetylcholine receptor (AChR) were used to prepare mouse hybridomas. Culture supernatants were directly screened in two different assays for recognition of mammalian muscle AChR. In one assay, unfixed, cryostat sections of mouse diaphragm were incubated with supernatants followed by FITC-conjugated second antibody (goat anti-mouse Ig). Localization to endplates was verified by visualization of AChR-rich regions with rhodamine-conjugated α-bungarotoxin (BTX). In the second assay, candidate supernatants were tested for immunoprecipitation of ^{125}I-BTX-AChR complex prepared by detergent extraction of a mouse muscle cell line (BC3H1) prelabeled with ^{125}I-BTX. Five of the hybridomas that were positive in both assays have been characterized in further detail. The monoclonal antibodies (mAbs) obtained from these hybridomas immunoprecipitate ^{125}I-BTX-AChR complex from Torpedo, BC3H1 cells, or embryonic chick muscle. By indirect immunofluorescence, the mAbs label the cell surface of fixed BC3H1 cells as well as the clusters of AChR on fixed, cultured chick myotubes.

The subunit specificity of the mAbs has been studied by electrophoretically transferring resolved AChR subunits from denaturing gels onto a nylon membrane, "Zeta-Bind" (ZB), followed by immunodetection. Using partially purified Torpedo AChR as a standard, the α-subunit of the AChR was identified by the direct binding of ^{125}I-BTX to as little as 20 ng of the immobilized polypeptide. This binding could be competed with d-tubocurarine. With analogous techniques, it has been determined that two of the five mAbs bind to the α-subunit of Torpedo AChR as visualized with ^{125}I- or alkaline phosphatase-conjugated goat anti-mouse IgG. The other three mAbs do not appear to recognize denatured receptor subunits.

AChR from BC3H1 cells was partially purified by an immunoaffinity procedure, subjected to LDS-electrophoresis and transferred onto a ZB membrane. The electrophoretically resolved α-subunit of BC3H1-derived AChR could be visualized by ^{125}I-BTX binding. This same procedure was used to show that a mAb, which bound the Torpedo-derived α-subunit, also bound the α-subunit of mouse BC3H1 cells. This approach will be of considerable use in further comparative studies of AChR derived from various sources.

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91.8 IDENTIFICATION OF POLYPEPTIDES ASSOCIATED WITH α-BUNGAROTOXIN BINDING TO NEURONAL MEMBRANES. H. Betz*, D. Graham* and H. Rehm*. (SPON: ENA). Max-Planck-Institute for Psychiatry, Department of Neurochemistry, 8033 Martinsried, Germany.

The nicotinic acetylcholine receptor ligand α-bungarotoxin (M_r = 8,000) binds with high affinity to a membrane glycoprotein in both the vertebrate and invertebrate central nervous system. Here we report different approaches to identify the polypeptides associated with this neuronal α-toxin receptor: i) ^{125}I-α-bungarotoxin bound to membrane fractions or monolayer cultures of chick retina was cross-linked to its binding site by using glutaraldehyde, or the photoactivable bifunctional reagent N-succinimidyl-6 (4'-azido-2'-nitrophenyl-amino)-hexanoate. Electrophoretic analysis of the cross-linked membrane proteins revealed ^{125}I-α-bungarotoxin-polypeptide adducts of apparent molecular weights of 63,000, 43,000 and 33,000. ii) Affinity purification of the α-bungarotoxin binding protein from detergent extracts of [^{35}S]methionine-labeled retina cultures identified one major polypeptide with an M_r = 57,000. iii) Indirect immunoprecipitation from detergent extracts of [^{35}S]methionine-labeled rat pheochromocytoma cells (PC 12) gave evidence for a specific co-precipitation of α-bungarotoxin with three polypeptides (M_r = 57,000, 34,000 and 25,000). These data suggest that polypeptides of M_r = 56,000, 34,000 and 25,000 (± 3,000) are located at or close to the α-bungarotoxin binding domain of the putative neuronal nicotinic acetylcholine receptor.

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91.9 MONOCLONAL ANTIBODY (mcab) TO THE α-BUNGAROTOXIN (αBgTx) BINDING SITE OF TORPEDO ACETYLCHOLINE RECEPTOR (T-AcChR) MODIFIES CHOLINERGIC LIGAND BINDING TO SOLUBILIZED AND MEMBRANE-BOUND AcChR. Mirta Mihovilovic* and David P. Richman*(SPON: R. Dinerstein). Dept. of Neurology, The University of Chicago, Chicago, IL 60637

We have produced a library of anti-AcChR mcabs by the hybridoma technique involving fusion of spleen cells from rats immunized with purified T-AcChR to the cell line SP/20-Ag14. One of these antibodies, mcab 247, is unable to bind to AcChR-αBgTx complexes as shown by hemagglutination of AcChR-coated red blood cells and by a radioimmunoprecipitation assay. To investigate the effect of mcab 247 upon the ligand binding properties of membrane-bound and solubilized T-AcChR, DEAE-cellulose-purified mcab and purified T-AcChR preparations were used. Analysis of AcChR-mcab 247 mixtures by radioimmunoprecipitation assay and sucrose gradient sedimentation shows that one mcab binds one T-AcChR molecule (two αBgTx binding sites) while [^{125}I]αBgTx binding is inhibited up to a maximum of 50%, even in the presence of mcab excess. The αBgTx binding inhibition is time dependent and, at the equivalence point, is overcome after 18 hrs incubation.

Cholinergic ligand binding was investigated by the inhibition of initial rate of binding of [^{125}I]αBgTx to AcChR-mcab complexes. The data indicate that: 1) cholinergic ligands (carbamylcholine, benzoquinonium and d-tubocurarine) bind to AcChR-mcab 247 complexes but their rate of dissociation from the complex is reduced; 2) the affinity of d-tubocurarine for the AcChR-mcab 247 complex is reduced by a factor of 10; 3) the high affinity binding of benzoquinonium ($K_d \approx 10^{-7}$M) is blocked by mcab 247, while the low affinity site ($K_d \approx 10^{-4}$M) is not affected; 4) carbamylcholine is able to desensitize the membrane bound AcChR in the presence of mcab 247; 5) a control anti-T-AcChR mcab that is not directed against the αBgTx binding sites has no effect on ligand binding.

These results indicate that mcab 247 is able to recognize a ligand binding site on the AcChR molecule specific for the high affinity binding of benzoquinonium. The maximum inhibition of αBgTx binding of 50%, the stoicheometry of mcab binding, and our previous data in which the presence of benzoquinonium diminishes mcab 247 binding to T-AcChR are consistent with the notion that mcab 247 is able to bind only one of the two αBgTx binding sites in the T-AcChR molecule.

We conclude that antibodies directed against the toxin binding site of acetylcholine receptor not only could directly affect its function leading to impairment of neuromuscular transmission by: a) decreasing ligand affinity for the AcChR molecule, b) directly blocking ligand binding and/or c) freezing a given conformation of the AcChR, but as well can be used as "fine" probes of the two αBgTx binding sites of the AcChR molecule.

91.10 CHARACTERISATION AND PARTIAL PURIFICATION OF A PUTATIVE NICOTINIC ACETYLCHOLINE RECEPTOR FROM LOCUST CNS. M. T. Filbin*† and G. E. Lunt* (SPON: D. R. Burt). Dept. of Biochemistry, University of Bath, Bath, BA2 7AY, England.

Specific [^{125}I]α-bungarotoxin ([^{125}I]α-BgTx) binding was defined as the binding displaced by 10^{-3} M d-tubocurarine. Specific [^{125}I]α-BgTx binding to a membrane fraction of locust supra-oesophageal ganglia was measured by ultracentrifugation and increased linearly with protein, saturated at concentrations of [^{125}I]α-BgTx greater than 5 nM (K_d = 1.38 nM, B_{max} = 1.18 pmol/mg protein), dissociated in a biphasic manner and was preferentially inhibited by nicotinic cholinergic ligands. After treatment of the membrane fraction with the covalent affinity label [^{3}H]-4-(N-maleimido)-benzyltrimethylammonium iodide ([^{3}H]-MBTA) followed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), most of the radioactivity was recovered as a single peak, corresponding to an M_r of 58,000. The binding characteristics (K_d and B_{max}) of the membrane fraction did not change significantly upon solubilisation in Triton X-100. Up to a 1000-fold purification of the putative nicotinic acetylcholine receptor (nAChR) was achieved after affinity chromatography on immobilised α-BgTx. From equilibrium binding studies no significant change in the K_d (1.71 nM) was observed after this purification step, but determination of the k_{-1} showed dissociation of [^{125}I]α-BgTx to be monophasic. The partially purified putative nAChR sedimented as a single component after centrifugation in a sucrose density gradient (14.2S; M_r 400,000-450,000), and SDS-PAGE showed two major protein bands of M_r's 60,000 and 41,000. No acetylcholinesterase activity was detected in this partially purified receptor fraction. It is suggested, therefore, that a nAChR from locust CNS has been partially purified which bears a closer similarity to the nAChR reported present in housefly and fruitfly heads and mammalian CNS than to the nAChR from the vertebrate neuromuscular junction or electric fish electroplax.

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91.11 ANTIBODIES THAT DISTINGUISH BETWEEN DEVELOPMENTALLY DIFFERENT FORMS OF MUSCLE ACETYLCHOLINE RECEPTORS P. Gorin, L. Silberstein, S. Tzartos, J. Lindstrom and Z. W. Hall Dept. Physiology, Univ. California, San Francisco, CA. 94143 and The Salk Institute, San Diego, CA. 92138

Acetylcholine receptors (AChR) in embryonic and denervated muscle located at extrajunctional sites (EJR) can be distinguished from AChR at the normal adult neuromuscular junction (JR) by immunological (1,2), electrophoretic (3), and other properties. We have identified two antibody preparations that bind selectively to rodent muscle EJR or JR in situ. The first is a serum from a patient with myasthenia gravis which, in rodent muscle, shows an absolute specificity for EJR and which is directed at determinants associated with the binding site for α-bungarotoxin. Serum antibodies inhibited by approximately 40% the binding of radiolabeled α-Butx to EJR in situ on aneural myotube cultures of C_2 cells (derived from C_3H mice) (4). Similarly the antibodies inhibited toxin binding to detergent-solubilized and partially purified EJR from C_2 cells or denervated rat leg muscle. As no antibody binding was detected with EJR-toxin complexes, the serum antibodies appear to be specific for determinants associated with the toxin binding site on EJR. The second specific antibody is a rat monoclonal antibody (mAb) raised against the β-subunit of Torpedo AChR which shows specificity for JR in situ. Fluorescent anti-rat antibodies were used to demonstrate mAb binding at adult C_3H mouse and rat muscle endplates, whereas no binding was detected to AChRs in clusters on C_2 myotubes in culture. However, the monoclonal antibody bound detergent-solubilized and partially purified toxin-receptor complexes from both C_2 myotubes and adult mouse muscles equally well. Thus the specificity of the monoclonal antibody for JR appears to involve determinants other than the α-Butx binding site and to reflect conformational or structural differences that are present on AChR in the membrane but that are not preserved after solubilization. The EJR- and JR-specific antibodies will be used to study the appearance of the two types of receptors in developing and denervated muscle and to correlate immunological properties of the AChR with other properties that change during development.

(1) Almon and Appel (1975) BBA 393, 66-77; (2) Weinberg and Hall (1979) PNAS 76, 504-508; (3) Brockes and Hall (1975) Biochem 14, 2100-2106; (4) Yaffee and Saxel (1977) Nature 270, 725-727.

91.12 EFFECT OF GONADAL STEROIDS ON [^{125}I]αBUNGAROTOXIN LABELING OF THE SUPRACHIASMATIC NUCLEUS OF THE RAT HYPOTHALAMUS. M. M. Miller, R. B. Billiar, and J. Silver. (SPON: K. Alley). Dept. of Reprod. Biol. and Anatomy, Case Western Reserve Univ., Cleveland, OH 44106.

Receptor binding in the hypothalamus of the rat has been investigated by the use of (^{125}I)alpha bungarotoxin (αBTX), a radioligand specific for nicotinic cholinergic receptors. Previous work in our laboratory using infusion of the α-neurotoxin into the third ventricle of ovariectomized female rats (N=22), normally cycling rats (N=12), or normal male rats (N=4) followed by light microscopic autoradiography has demonstrated that the supraoptic, periventricular, arcuate, premamillary, and mamillary nuclei of the hypothalamus consistently label. In intact females and males, the suprachiasmatic nucleus (SCN) was also consistently labeled. In marked contrast, when the SCN of the chronically oophorectomized female was examined, the binding of (^{125}I)αBTX was markedly reduced or not present (Miller et al., Br. Res., 1982, In press). The present work demonstrates that, in contrast to gonadectomy in the female rat, following chronic castration of adult males (4 to 5 wks) (N=8), (^{125}I)αBTX still binds to the SCN in a manner comparable to that of the intact males and females. We next tested whether the ovarian effect on αBTX labeling of the SCN could be duplicated by estrogen replacement. For this study, a total of 3200 serial sections from the rostral to caudal extent of the SCN were examined. When females are provided with a constant dose of estrogen (subcutaneous silastic capsule implant, 0.5 or 1.0 mm for 5 weeks) (N=7) at the time of ovariectomy, (^{125}I)αBTX binding to the SCN was maintained. If animals are ovariectomized for 3 wks, and estradiol capsules are implanted subsequently for 2 weeks, binding of the neurotoxin to the SCN was largely restored (N=6). On the other hand, if chronically oophorectomized animals (4 wks) are then supplied with estrogen for only one week (N=2), (^{125}I)αBTX binding is incompletely restored. The SCN of oophorectomized controls (N=3) again did not demonstrate αBTX binding. Thus, in the ovariectomized rat the presence of circulating estrogen for sufficient duration can both maintain and restore the ability of the SCN to bind αBTX. The labeling of the SCN in females without ovarian hormones is markedly different from that of intact females, intact males, or castrated males, and it appears that estrogen, either directly or indirectly, is necessary for the binding of αBTX to the SCN of the adult female rat. (Supported in part by NIH Training Grant HD-07120, P-50 Center Grant HD-07640 and NS 15731 (JS)).

91.13 ANTIBODY AGAINST ACETYLCHOLINE RECEPTOR MEASURED BY AN ENZYME-LINKED IMMUNOSORBENT ASSAY. C.L. Hinman[1]*, R.A. Hudson[2]* and H.C. Rauch[1]. (Spon: D. Drescher). Depts. Immunol./Microbiol. and Biochemistry[2], Wayne State Univ. Sch. of Med., Detroit, MI 48201.

An alkaline-phosphatase enzyme-linked immunosorbent assay (ELISA) has been developed for measurement of antibody against acetylcholine receptor [Ab(AcChR)]. The assay is highly reproducible, sensitive, and requires small quantities of immunologic reagents. Relative measurements of antibody concentration by this method are proportional to those determined by radioimmunoassay (RIA). This ELISA was originally developed using purified Torpedo californica receptor (T-AcChR) and serum from rabbits immunized with that or other antigens. Parameters optimized included reactant concentrations, incubation times, and temperatures. AcChR was bound to the wells of microtitration plates by previously adsorbed alpha-bungarotoxin (BTx) applied at 1 µg/ml. Higher coating concentrations of BTx led to increased variability due to the increased rate of substrate conversion. Saturation of BTx occurred for T-AcChR greater than 10 pM/ml. Enzyme-conjugated second antibody was also used at saturating levels (250 ng/ml for the rabbit system). Kinetic studies indicated that immobilization of enzyme by binding of covalently linked second antibody to a heterogeneous set of serum antibodies specific for different AcChR determinants does not alter the noncooperative enzymatic conversion of substrate.

Plots of absorbance versus log serum antibody concentration were sigmoidal, comparable to RIA results. Parallel linear regions of those curves allowed relative levels of Ab(AcChR) to be compared among subjects.

The use of human AcChR allows measurement of Ab levels from the sera of patients with myasthenia gravis. Those results are presented and compared with RIA measurements from the same patients. The ELISA is recommended because it offers advantages of convenience, reagent stability, safety, and lower equipment costs than the RIA.

This research was supported in part by NIH grants AI-07118 and NS-14491 and by a grant from the Muscular Dystrophy Assoc.

91.14 β-ADRENERGIC RECEPTOR ACTIVATION INCREASES ACETYLCHOLINE RECEPTOR NUMBER IN CULTURED SKELETAL MUSCLE MYOTUBES. James C. Blosser. Pennwalt Pharm. Corp., Rochester, NY 14623 and Baylor College of Medicine, Houston, Texas 77030.

Primary cultures of embryonic muscle have proved to be a useful model in understanding the biochemical processes which control the number and metabolism of nicotinic cholinergic (Ach) receptors. We previously showed that cAMP increases the total number of Ach receptors and receptor insertion rate into surface membrane of cultured chick embryonic muscle (J. Biol. Chem. 255: 1235, 1980). The presence of the β_2-adrenergic receptor-linked adenylate cyclase system in skeletal muscle suggested that cAMP effects on Ach receptor number may be under hormonal control. Cultures of chick myotubes were treated with the β-adrenergic agonist isoproterenol and Ach receptor numbers were quantified by ^{125}I-α-bungarotoxin binding. ℓ-Isoproterenol increased the number and insertion rate of Ach receptors into surface membrane without altering the rate of degradation. The effect of isoproterenol was dose dependent and stereospecific, the d-isomer having less than 1% the potency of the ℓ-isomer. The response to ℓ-isoproterenol could be abolished by the β-adrenergic antagonist alprenolol but was unaffected by the α-adrenergic antagonist phentolamine. Other catecholamines stimulated increases in Ach receptor number and followed an order of potency isoproterenol > epinephrine > nonepinephrine, consistant with a β_2 adrenergic receptor mediated process. It was shown previously that cholera toxin, an irreversible activator of adenylate cyclase, increases receptor numbers by increasing the rate of receptor insertion into surface membrane. When ℓ-isoproterenol and cholera-toxin were added together to myotubes, the increase in Ach receptor levels was no greater than that produced by maximally active concentrations of either agent added alone. These results suggest that isoproterenol and cholera toxin share a common mechanism involving cAMP which is responsible for the increase in Ach receptors. Hormonal regulation of Ach receptors in muscle by a β-adrenergic receptor mediated process may be of importance in certain physiological or pathological states such as development or denervation. (Supported in part by NSF grant BNS7914115 and a MDA grant).

91.15 A BINDING ASSAY FOR THE LEVAMISOLE RECEPTOR OF THE NEMATODE CAENORHABDITIS ELEGANS. J. A. Lewis and J. T. Fleming*. Div. of Biological Sciences, Univ. of Missouri, Columbia, MO 65211.

We have developed a glass fiber filter binding assay for the levamisole receptor, a putative acetylcholine receptor of the nematode Caenorhabditis elegans. Levamisole is a nicotine-like drug neurotoxic to nematodes. Mutants can readily be selected for resistance to the muscle contracting effects of levamisole and these levamisole-resistant mutants also prove to be deficient in their response to all cholinergic agonists effective on the wild type nematode (Genetics 95: 905, 1980; Neuroscience 5: 967, 1980). Hypothesizing that at least some of the mutants might lack a receptor responsible for the wild type response to levamisole, we made a radioactive ligand for receptor binding assays. We iodinated meta-aminolevamisole (MAL) and then catalytically dehalogenated the iodinated compound with tritium gas (Lewis and Paterson, unpublished). The purified [^{3}H] MAL has the same chromatographic behavior and high biological potency of authentic MAL and has a specific radioactivity of ~20 Ci/mmole. With [^{3}H] MAL, a saturable, high affinity binding activity can be detected in wild type extracts (K_D ~20-30 nM). Wild type worms contain about 0.5 pmole of binding activity per gm wet weight, with a minimum assay sensitivity of 6 x 10^{-16} mole. Levamisole derivatives and cholinergic agonists inhibit [3H] MAL binding in vitro with the same relative potencies with which they cause muscle contraction of the living worm. A preliminary screening of mutants extremely resistant to levamisole reveals several different mutants with little or no binding activity. Altogether there are about 10 genetic loci identifiable by levamisole resistance that are candidates for genes required to produce a fully functional levamisole receptor. We think it likely that some of these genes will prove to be receptor structural genes while others may be genes required for receptor regulation, processing, or localization. The potential for studying more than just the structural genes of a receptor makes this system an interesting one to study.

92.1 MUSCARINIC AND NICOTINIC RECEPTORS IN THE SAME STRIATED MUSCLE FIBER ARE INDEPENDENTLY REGULATED. R. Núñez*, G. Pilar, and I. McLennan*, Physiology Section, Biological Sciences Group, Univ. of Connecticut, Storrs, CT 06268.

During development, the chick iris is activated initially via muscarinic receptors. Later response to nerve stimulation is mediated by nicotinic junctions (Núñez, et al, Abstracts for Neurosci. 1980). In this new series of experiments, the change in the mode of synaptic activation of the iris muscle was studied to ascertain the distribution of different receptors, as well as their dependence on innervation.

In isolated iris from 2 week-old chicks, it was found that the response elicited either by nerve stimulation or ACh superfusion resulted in activation of all the muscle fibers. When repetitive nerve stimulation was used (70Hz), a fast contraction was maintained during the whole stimulation period. After αBTX application (1 ug/ml), nerve stimulation failed to activate the muscle fibers. However, addition of ACh (1×10^{-4}g/ml) to the bathing solution elicited a contracture similar to the one induced by nerve activation. When atropine (0.2uM) was added to the bath, all responses were eliminated. When recording intracellularly from iris fibers, a microinjection (2ul) of ACh (0.25ug) produced a fast initial depolarization followed by a slow, long-lasting depolarization. Membrane resistance was reduced during the initial depolarization, whereas it was increased during the subsequent depolarization. The initial increase in conductance, as well as the initial depolarization, were abolished by αBTX, but the slow, long-lasting depolarization and the reduced conductance were still present.

Specific binding of ^{3}H-QNB and ^{125}I-αBTX was done in control and denervated iris muscles. In response to denervation, there was a two-fold increase in nicotinic receptor binding (498.5 ± 84.5 vs 981.2 ± 301.1 (5) Fmol/mg protein) whereas the muscarinic binding remained unchanged (24.01 ± 6.36 vs 18.46 ± 5.33 (5) Fmol/mg protein). It is concluded: a) both receptors are present in the same muscle fiber; b) the nicotinic receptors are mainly confined to the junctional membrane, whereas the muscarinic are extrajunctional. Furthermore, because of the early appearance of the muscarinic receptor, later appearance of the nicotinic receptor, and the different response of each to denervation, it is hypothesized that the nicotinic and muscarinic receptors are independently regulated in the same muscle cell. Partially supported by NIH MS 10330, Muscular Dystrophy Association of America and the Univ. of Conn. Research Foundation.

92.2 AUTORADIOGRAPHIC LOCALIZATION OF M1 AND M2 MUSCARINE RECEPTORS IN THE RAT BRAIN. D.C.Mash and L.T.Potter. Department of Pharmacology, University of Miami School of Medicine, Miami, FL 33101

(-)^{3}H-Quinuclidinyl benzilate (QNB) and similar ligands are known to be suitable for visualizing muscarine receptors by autoradiography. Two facts have prompted us to re-evaluate the localization of these receptors. First, it is clear from in vitro work that muscarine receptors form high affinity complexes with endogenous agonist and a regulatory protein, and that dissociation of the agonist is often so slow as to mask a significant fraction of the total receptors, especially those of a type common in the brainstem (M2). "Presoaking" membranes in 50 mM sodium phosphate-10 mM EDTA for 30 min at 0-4°C overcomes this problem (Potter, Luber-Narod & Wohlberg, Neurosci. Abstr. 1982). Second, there are two different types of receptors, one of which (M1) is common in the forebrain and is selectively blocked by pirenzepine, and the other of which (M2) is selectively occupied by carbachol (Luber-Narod & Potter, Neurosci. Abstr. 1982).

Most sections (25 μ) of unfixed rat brain were presoaked in 10 mM EDTA. Some were fully saturated with 1 nM ^{3}H-QNB in 50 mM phosphate-1 mM EDTA in 30 min at 20°C, while others were incubated in sufficient pirenzepine or carbachol to diminish binding of QNB to M1 or M2 sites by 80-90%. All but a few percent of unbound QNB was then removed by 3 5-minute washes at 4°C. Blanks contained 1 μM (±) QNB.

As expected, carbachol-resistant and pirenzepine-sensitive receptors (M1) were the most prevalent in the forebrain; particularly dense labeling was apparent in the caudate-putamen, nucleus accumbens, and laminae 1-4 of the cerebral cortex. Within the brainstem, M1 sites were notable in the reticular formation. Carbachol-sensitive and pirenzepine-resistant sites (M2) were more evident in the cerebellar cortex and many brainstem nuclei, including those of the trigeminal (V) and hypoglossal (XII) nerves, nucleus tractus solitarius, medial vestibular nucleus, and the lateral cuneate nucleus. Within the forebrain, M2 sites were notable in lamina 4 of the cortex and the nucleus tractus diagonalis. Sections not presoaked in EDTA showed, in the forebrain, a pattern similar to that seen in carbachol.

M1 and M2 receptors may have evolved at different times and may, in part, differ in location pre- and post-synaptically. Our anatomical data and biochemical evidence for greater M2 than M1 coupling to a regulatory protein, suggest the possibility of a 3rd, functional difference. M2 receptors may be used where one agonist is sufficient for most postsynaptic responses; then 1:1 receptor:effector coupling is desirable. M1 receptors may be used at more integrative neurons where many different receptors produce varied responses via fewer effector proteins.

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92.3 TWO TYPES OF MUSCARINE RECEPTORS FROM THE RAT BRAIN, IN SOLUTION. L. Kalinoski and L.T. Potter. Department of Pharmacology, University of Miami School of Medicine, Miami, Florida 33101.

Two populations of muscarine receptors (M1 and M2) have been identified in membranes from the rat brain according to their affinity for (-)^{3}H-quinuclidinyl benzilate (QNB), the antagonist, pirenzepine, and the agonist, carbachol (Luber-Narod & Potter, Neurosci. Abstr. 1982). The properties of these sites have been examined further in solution, utilizing an assay (Kalinoski & Potter, Neurosci. Abstr. 7, 498, 1981) which permits kinetic as well as equilibrium analyses.

Membranes were prepared from the rat forebrain and brainstem in 50 mM sodium phosphate-1 mM EDTA, pH 7.4, so as to uncouple receptors from endogenous agonist. Receptors were then dissolved and assayed in digitonin in the same buffer. Recovery varied from 55-75%, so results are not quantitative with respect to original receptor prevalence. Measurements of the association rate for QNB yielded similar values for forebrain and brainstem sites. As in membranes, QNB dissociated more rapidly from brainstem than forebrain sites, and kinetic Kd values were different (forebrain ∿ 9 pM; brainstem ∿ 25 pM). Inhibition experiments demonstrated that pirenzepine was selective for sites predominant in the forebrain (M1), while carbachol was selective for those in the brainstem (M2).

We conclude that the binding properties of M1 and M2 muscarine receptors are effectively the same in solution as in membranes.

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92.4 TWO TYPES OF MUSCARINE RECEPTORS (M1 AND M2) IN MEMBRANES FROM THE RAT FOREBRAIN AND BRAINSTEM. J. Luber-Narod and L.T. Potter. Department of Pharmacology, University of Miami School of Medicine, Miami, Florida 33101.

The binding properties of (-)^{3}H-quinuclidinyl benzilate (QNB) to muscarine receptors were studied under a variety of conditions which prevent or reverse the formation of "ternary" agonist-receptor complexes with guanine nucleotide binding protein (Flynn, Kalinoski, Luber-Narod & Potter, Neurosci. Abstr. 1982). When such conditions are optimal, all receptor sites are available for assays, and agonists as well as antagonists bind to only a single affinity state for a given receptor type. Unselected membranes were prepared in 50 mM sodium phosphate-10 mM EDTA, pH 7.4, to dissociate ternary complexes containing endogenous agonist (Potter, Luber-Narod & Wohlberg, Neurosci. Abstr. 1982). Assays with an excess of a saturating concentration of QNB were carried out at pH 7.4 in 50 mM sodium phosphate-1 mM EDTA or in 20 mM Tris-1 mM $MnCl_2$ ± 0.1 mM GppNHp. In EDTA, QNB bound to and dissociated from brainstem receptors faster than forebrain sites; Kd values determined from kinetic and equilibrium data were similar (forebrain ∿ 10 pM; brainstem ∿ 20 pM). In Mn ± GppNHp, QNB binding and dissociation were slower, especially for brainstem receptors, and kinetic Kd values were halved. Inhibition experiments with pirenzepine demonstrated that most receptors in the forebrain, and especially in the hippocampus, had higher affinity for this antagonist than did most sites in the brainstem. For the agonist, carbachol, in EDTA, the reverse was true, the brainstem receptors showing higher affinity. Alkaline extraction of membranes ± mercaptoethanol, under conditions which extract a guanine nucleotide binding protein associated with receptors which activate adenylate cyclase, yielded carbachol affinities like those seen in EDTA alone. Assays in Tris-Mn + GppNHp gave the same result. Computer analyses indicate two types of sites in EDTA with approximate carbachol dissociation constants of 1 and 30 μM in each region of the brain.

We define M1 receptors as those showing ∿ 2-fold selectivity for QNB and ∿ 30-fold selectivity for pirenzepine in EDTA. These comprise about 75% of the sites in the hippocampus and about 33% of the sites in the brainstem. M2 receptors show ∿ 30-fold selectivity for carbachol in EDTA, couple more extensively than M1 in high-affinity agonist complexes in Mg or Mn, and comprise the remainder of the total sites - about a third of all the receptors in the whole rat brain.

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92.5 EDTA SIMPLIFIES AGONIST- AND ANTAGONIST-BINDING PHENOMENA, AND HELPS RESOLVE DIFFERENT TYPES OF RECEPTORS. L.T. Potter, J.Luber-Narod and C.J. Wohlberg* (SPON: D. Liskowsky). Department of Pharmacology, Univ. of Miami School of Medicine, Miami FL 33101.

Muscarine receptors in the rat brain were studied by binding assays with (-)^{3}H-quinuclidinyl benzilate (QNB). When membranes were prepared and assayed in 20 mM Tris (pH 7.4) plus 10 mM Mg or 1 mM Mn ions, fewer receptor sites were found than when membranes were prepared or assayed in Tris or phosphate buffer plus 1-10 mM EDTA, especially in brain regions like the brainstem where agonist-receptor complexes sensitive to GppNHp are most apparent (Flynn, Kalinoski, Luber-Narod & Potter, Neurosci. Abstr. 1982). Mg reduced assayable receptors more than Mn, e.g. brainstem sites for QNB were 30 and 60% of the total seen in EDTA, respectively. When brainstem receptors were saturated with carbachol in Mg or Mn, and then assayed in the absence of free agonist, only 15 and 30% of the total sites were seen. Carbachol dissociated very slowly during incubation at 37 in Mg or Mn, freeing QNB sites. Masked sites are clearly due to prolonged agonist occupation rather than to very high agonist affinity, since Kd values for QNB and carbachol differ $\sim$ 10,000-fold. Incubation was also necessary to fully dissociate agonists in 1 mM EDTA. The simplest procedure for uncoupling was "presoaking" membranes in homogenates on ice in 50 mM sodium phosphate-10 mM EDTA, pH 7.4, for 30+ minutes. The same procedure is suitable for preparing tissue sections for autoradiography (Mash & Potter, Neurosci. Abstr. 1982). When returned to Mg or Mn, receptors showed the usual 3-4 affinity states for carbachol. In contrast, inhibition curves obtained with membranes in 1 mM EDTA disclosed only two receptor classes with lower agonist affinity. These are clearly separate receptors rather than coupling states (Luber-Narod & Potter, Neurosci. Abstr. 1982).

Thus EDTA has several uses. (1) For receptor preparation, EDTA dissociates complexes of endogenous agonist-receptor-guanine nucleotide binding protein, facilitating subsequent study of all sites in any medium. Incubation is unnecessary, protease activity is inhibited, enzymes like acetylcholinesterase are dissolved, and the cost and effects of a guanine nucleotide are avoided. (2) In EDTA, agonists, like antagonists, can be used to distinguish different receptor types, without the confusing presence of two affinity states for each receptor. (3) During inhibition studies involving agonists, equilibrium is achieved more rapidly in EDTA than in Mg or Mn.

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92.6 EVIDENCE FOR GUANINE NUCLEOTIDE-SENSITIVE REGULATORY PROTEIN ASSOCIATED WITH MUSCARINE RECEPTORS IN MEMBRANES AND IN SOLUTION. D.D. Flynn*, L.Kalinoski, J. Luber-Narod and L.T. Potter. Department of Pharmacology, University of Miami School of Medicine, Miami, Florida 33101.

(1) When membranes are prepared from the rat brain in 20 mM Tris-1 mM $MgCl_2$ or $MnCl_2$, and muscarine receptors are assayed with (-)^{3}H-quinuclidinyl benzilate (QNB), fewer sites are found than when assays are carried out in EDTA (Potter, Luber-Narod & Wohlberg, Neurosci. Abstr. 1982). Addition of 0.1 mM GppNHp to the Tris buffer restored the missing sites. This phenomemon was more apparent with brainstem than forebrain receptors.

(2) Inhibition of the binding of QNB to membranes in Tris-Mn by varying concentrations of the agonist, carbachol, involves at least three (probably four) affinity states of muscarine receptors in both the forebrain and brainstem. In 0.1 mM GppNHp high affinity carbachol sites were greatly reduced, and only two affinity states remained; these can be attributed to separate kinds of receptors (Luber-Narod & Potter, Neurosci. Abstr. 1982). The effect of GppNHp was not removed by washing membranes in EDTA. The shift in agonist affinity was considerably greater for brainstem receptors than for those in the forebrain, apparently because the former couple more extensively with a GppNHp-sensitive component of membranes.

(3) When membranes were dissolved in digitonin in 50 mM sodium phosphate-1 mM EDTA, and then assayed in Tris-Mn, carbachol showed high affinity inhibition of the binding of QNB. Both 0.1 mM GppNHp and 1 mM EDTA sharply reduced carbachol affinity.

(4) Receptor complexes from membranes exposed to carbachol before solubilization in digitonin sedimented faster in sucrose gradients than receptors without agonist or receptors also exposed to 0.1 mM GppNHp or EDTA.

These observations suggest the presence of high agonist-affinity complexes containing receptor, agonist and a guanine nucleotide binding protein. These appear to be dependent upon the presence of Mg or Mn ions. Both GppNHp and EDTA uncouple these complexes, resulting in lower agonist affinities.

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92.7 CHARACTERIZATION AND INITIAL PURIFICATION OF MUSCARINIC ACETYLCHOLINE RECEPTORS. Hemin R. Chin, Mary Lambert* and William L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

In our initial efforts to purify muscarinic Ach receptors, we have taken two different, but complementary approaches. The first is to irreversibly label membrane-bound receptors with the muscarinic antagonist 3H-propylbenzylcholine mustard (PrBCM) and to follow the specific radioactivity in subsequent solubilization and purification steps. 3H-PrBCM at nanomolar concentrations labeled ~60% of muscarinic receptors in mouse and calf brain membranes with specific to nonspecific binding ration of 1.6. Sedimentation of 3H-PrBCM-labeled muscarinic receptors from mouse brain on 5 - 30% sucrose gradient coincided with that of 3H-QNB-bound, digitonin solubilized receptors on sucrose density gradients. 3H-QNB:receptor complexes from mouse neuroblastoma-rat glioma hybrid cells (NG108-15) gave sedimentation coefficient of 8.4 on the same sucrose gradient. Molecular weight of 3H-PrBCM-labeled mouse brain receptors was estimated to be 83,000 ± 1,300 on 7½% SDS polyacrylamide gels. Since solubilization of the membranes with digitonin yielded poor recovery of solubilized proteins (< 35%), we have tested other detergents for their ability to solubilize both muscarinic receptors and membrane proteins. A novel nonionic detergent, CHAPS, which was first synthesized by Hjemeland (P.N.A.S. 77: 6368, 1980), was most effective, solubilizing 50% of the receptors and 60% of total membrane proteins. In the same preparation, digitonin was able to solubilize 25% of the receptor protein and 33% of total proteins. The solubilized receptors with 3H-PrBCM label were loaded onto DEAE-Sepharose column for initial purification. Our preliminary results indicated that specifically labeled receptors were eluted as a single radioactivity peak with 0 - 500 mM NaCl gradient. Specifically labeled receptors could be completely separated from nonspecifically labeled proteins. Our second approach in purifying muscarinic Ach receptors involves use of muscarinic affinity chromatography. Tritiated p-aminoatropine has been synthesized via its nitro intermediate in preparation for its use as an affinity column ligand. Experiments are being carried out exploring these two alternative approaches for muscarinic receptor purification. (Supported by NIH grant NS15299 to WLK. Dr. Lambert was supported by NIH training grant HD-07068-04.)

92.8 DIFFERENTIATION OF POPULATIONS OF MUSCARINIC RECEPTORS IN RAT STRIATUM BY GALLAMINE ANTAGONISM OF [^{3}H]QNB BINDING. H. S. Blaxall* and R. W. Gardier*, (SPON: J. B. Lucot), Depts. of Biol. Sci., and Pharmacol. and Toxicol., Wright State University, Dayton, Ohio 45435.

Previous studies have indicated that gallamine could functionally discern subpopulations of muscarinic receptors in the superior cervical ganglion of the cat (Gardier, R. W. et al. J. Pharmacol. Exp. Ther. 204:46, 1978) and rat. These receptors were found to subserve two pathways, one being inhibitory and blocked by gallamine, the second being excitatory and insensitive to gallamine. Before further defining the sympathetic ganglionic receptors through ligand-receptor binding, it appeared prudent to establish the binding characteristics of gallamine in brain where muscarinic receptors have been subject to extensive investigation.

Striata were obtained from male rats selected at random from Fisher 344 and Sprague-Dawley strains. All tissues were used within 30 days of storage at -40°C. The experimental procedure was essentially that described by Yamamura, S. I. and S. H. Snyder (Proc. Natl. Acad. Sci USA 71:1725, 1974). A K_D of 0.25 nM was determined for L[^{3}H]-quinuclidinyl benzilate ([^{3}H]QNB) binding specifically to muscarinic receptors. The inhibitory concentration of atropine producing 50 percent inhibition of [^{3}H]QNB binding (IC_{50}) was 10nM, while complete inhibition occurred at 1μM. Parallel studies substituting gallamine for atropine gave an IC_{50} for gallamine of 1μM. However, gallamine in concentrations up to 1mM did not completely inhibit [^{3}H]QNB binding.

Since pilocarpine is an apparent specific agonist for muscarinic receptors in the ganglionic excitation pathway (Takeshige, C. and R. L. Volle, J. Pharmacol. Exp. Ther. 146:335, 1964) it was speculated to inhibit only that subpopulation of receptors insensitive to gallamine. However, pilocarpine demonstrated indiscriminate competitive binding capacity against [^{3}H]QNB, being similar to atropine but with 10^3 lesser affinity.

The different gallamine affinities for striatal muscarinic receptors confirms earlier studies by Ellis, J. and W. Hoss (Neurosci. Abstracts 6:254, 1980), and give promise that subpopulations of muscarinic receptors may be similarly identified in sympathetic ganglia where operational muscarinic pathways have been definitively described.

92.9 MULTIPLE STATES OF CARDIAC MUSCARINIC RECEPTORS. J.W. Wells,* H.-M. Wong,* T.W.T. Lee* and M.J. Sole* (SPON: P. Brawley). Faculty of Pharmacy and Department of Medicine, University of Toronto, Toronto, Canada M5S 1A1

(-)-N-[3H]Methylscopolamine (NMS) has been used to probe the binding properties of muscarinic receptors in left ventricular homogenates from the Syrian golden hamster. Assays were carried out at equilibrium in Krebs-Henseleit buffer at 30° using microcentrifugation to obtain the bound radioligand. A component of the binding is saturable and well described by a rectangular hyperbola. Analysis in terms of a uniform population of sites reveals an equilibrium dissociation constant of 0.39±.02 nM and maximal specific binding of about 70 pmol/g protein. Competitive studies with unlabelled drugs versus 1 nM [3H]NMS revealed a pattern typical of muscarinic receptors in various tissues. Hill coefficients were near one (0.93-1.00) for six antagonists, somewhat lower (0.82) for the partial agonist pilocarpine and lowest (0.65-0.73) for six full agonists. All drugs appear to compete for a common population of sites. With agonists, good descriptions of the data were obtained assuming multiple classes of sites differing in affinity for the drug. Carbachol, arecoline, oxotremorine-M and bethanechol revealed two classes. Methacholine and oxotremorine revealed three classes in some preparations; the third class constituted only about 10% of total specific binding and exhibited the highest affinity. Two observations indicate that the sites are interconvertible. Firstly, the relative size of each class varies markedly among agonists. Secondly, GMP-PNP but not AMP-PNP appears to convert sites of higher affinity to lower affinity. It is known that multiphasic binding could reflect a reversible association between a unitary receptor (R) and a nucleotide-specific G-protein (G) provided that two conditions are met: (1) the total amount of G is less than or comparable to the total amount of R, (2) agonists (A) show different affinities for R and for the RG complex. Four equilibria are involved: R+G=RG, AR+G=ARG, A+R=AR and A+RG=ARG. This model appears to be inadequate in the present circumstances. Simulated data obtained from the model generally are well described as a mixture of two, non-interconverting classes of sites. The log ratio of apparent affinities of A for each class correlates negatively with the apparent fraction of sites exhibiting lower affinity. In contrast, the experimental data exhibit a positive correlation (P≈.05). The effect of GMP-PNP strongly suggests that the binding of agonists reflects the influence of a G-protein. Inconsistencies between the simple form of the model and the experimental data suggest, however, that more than two states of the receptor may be involved. (Supported by the Medical Research Council and Heart Foundation, Canada, and by the US Public Health Service).

92.10 ADAPTATION OF MUSCARINIC RECEPTORS IN RAT RETINA AND BRAIN TO DIISOPROPYL FLUOROPHOSPHATE. Lynn Churchill, Dept. of Anatomy, University of Kansas Medical Center, Kansas City, KS 66103.

The rate at which muscarinic receptor binding decreased in response to diisopropyl fluorophosphate (DFP) was measured for rat retinas and six specific brain regions. The time course of the reduction in muscarinic receptor binding was documented by measuring ^{3}H quinuclidinyl benzilate (QNB) binding at various times during DFP treatment. DFP was administered at midday to Wistar rats intramuscularly at 9/10 LD_{50} for the first injection and one half of this amount on alternate days for further injections. Rats were killed by decapitation at 4h, 8h, and 24h after the first injection and 24h after subsequent injections. Binding of 1 nM ^{3}H QNB to muscarinic receptors in homogenates of retina and brain areas was determined by a filtration assay. Specific binding was defined as total binding minus binding in the presence of 10^{-5} M atropine.

In the retina, the rate at which muscarinic receptor binding decreased after DFP was faster and the amount of the decrease was larger than in the other brain regions analyzed. At 8h after DFP, retinal QNB binding to muscarinic receptors was reduced 20% relative to the controls. At 24h, retinal QNB binding was reduced 30%. After 2-3 injections of DFP, the binding was decreased to 40-45%, respectively. A student's t-test analysis revealed statistical differences at a probability level less than 0.02. In comparison, decreases in QNB binding in cerebral cortex were 17%, 24h after DFP and 25-30% after 2 to 3 injections. In corpus striatum and hippocampus, a 14-17% decrease was observed after 2-3 injections. In midbrain, brain stem, and cerebellum, no differences were observed after any of these various treatments.

To determine whether these modifications in QNB binding were due to changes in binding affinity or number of receptors, Scatchard analysis was conducted on homogenates of cerebral cortex after three injections of DFP or saline. The decrease in QNB binding was due to a reduction in the number of receptors rather than a decrease in the affinity of the receptor. These differences in muscarinic receptor binding for different brain areas may be in register with the rise in acetylcholine concentration during DFP treatment. Alternatively, the adaptive mechanisms of muscarinic receptors may be different in various brain regions.

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92.11 THE COUPLING OF MUSCARINIC CHOLINERGIC RECEPTORS TO INOSITOL PHOSPHOLIPID TURNOVER IN THE CNS. Stephen K. Fisher*, Paul D. Klinger* and Bernard W. Agranoff (SPON: G. J. Siegel). Neuroscience Lab, University of Michigan, Ann Arbor, MI 48109.

A ubiquitous response to the activation of muscarinic receptors is a selective increase in the incorporation of $^{32}P_i$ into phosphatidic acid (PhA) and phosphatidylinositol (PhI). In the CNS, this increased labeling reflects an increased diglyceride availability following the initial receptor-mediated breakdown of a phosphorylated derivative of PhI (J. Neurochem. 37:968). We have now investigated the relationship between the binding of the agonist to the receptor and resultant increase in PhA and PhI labeling, using a "light" nerve ending preparation from guinea pig cerebral cortex (J. Neurochem. 34:1231). The ability of cholinergic agonists to elicit increased labeling of PhA and PhI has been compared to their ability to displace [3H]QNB bound to nerve endings. Two groups of muscarinic agonists could be distinguished from their effects on PhA and PhI turnover. The addition of agonists in Group A (acetylcholine, carbachol, muscarine and methacholine) resulted in a large stimulation of both PhA and PhI labeling (200% or more of control), while agonists in Group B (bethanechol, pilocarpine, arecoline, and oxotremorine) were less effective (115-150% of control). Group B agonists block the stimulatory effects of Group A agonists, and can thus be termed "partial" agonists. All of the agonists could totally displace [3H]QNB bound to the nerve ending fraction. The ability of agonists to displace [3H]QNB binding was then evaluated for evidence of multiple binding sites using computer fits of the data to a non-cooperative two-site competitive inhibition model. The binding data from agonists in Group A best fitted the two-site model (35% sites high affinity, 65% low affinity), whereas Group B agonists best fitted a one-site model. The relationship between receptor occupancy and phospholipid turnover was examined for carbachol. Stimulation of PhA labeling, an early plasma membrane response, could be dissociated into high and low affinity components, using computer fits. However, only 12% of the inferred receptors were in the high affinity form. The dissociation constant for the low affinity state of the muscarinic receptor (109 μM) as determined from PhA labeling, was in good agreement with the value obtained from the binding data (77 μM). The results suggest that there is a close correlation between the ability of a given agonist to distinguish the high and low affinity sites or states of the muscarinic receptor, and its ability to increase PhA and PhI labeling. In the CNS, it is the low affinity state of the muscarinic receptor that is predominantly, if not exclusively, coupled to stimulated phospholipid turnover. (Supported by NIH Grant NS 15413.)

92.12 STRUCTURALLY SPECIFIC MODULATION OF ACETYLCHOLINE RECEPTOR BINDING BY VARIOUS BARBITURATES, B.A. Dodson* and K.W. Miller* (SPON: E.T. Hedley-Whyte). Depts. of Anesthesia and Pharmacology, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114.

There exists a pentobarbital binding site which appears to interact allosterically with the acetylcholine (ACh) binding site in acetylcholine receptor- (AChR) rich membranes from Torpedo (Miller, K.W. et al., BBRC 105:659, 1982). Here we examined the ability of a number of barbiturates to modulate the binding of [3H]-ACh. Receptor-rich membranes were prepared by differential and gradient centrifugation from homogenized electric organs from freshly killed Torpedo. Membranes were first pre-incubated with diisopropyl flurophosphate (10^{-4} M) to inactivate the AChesterase and then incubated with [3H] ACh under conditions expected to produce 30-50% AChR occupancy (typically 25 nM [3H] ACh and 15 nM ACh binding sites). Binding was measured by filtration assay.

Some barbiturates increased ACh binding, others had no effect, while the remainder decreased binding. Small changes in barbiturate structure caused large changes in ACh binding characteristics. Examples are: (1) pentobarbital decreased whereas thiopental increased ACh binding; (2) barbital had no effect but n-methylbarbital increased binding; and (3) 1,3-dimethylbutylbarbituric acid (DMBB) strongly increased binding, 1-methylbutylbarbital slightly decreased binding and 3-methylbutylbarbital strongly decreased binding. The above suggests that these barbiturates exert their effects by binding to a site or sites modulating ACh binding with an efficacy which shows considerably more structural specificity than would be expected from a non-specific membrane perturbation. The concentration dependence of a barbiturate from each class was examined. DMBB increased ACh binding ([3H] ACh bound/[3H] ACh free) by 60% with an apparent dissociation constant, $K_d \cong 45$ μM whereas amobarbital decreased binding by 70% with an apparent $K_d \cong 20$ μM. Both effects followed mass action. Secobarbital, which itself had no effect, antagonized the effect of amobarbital in a competitive manner, with an apparent $K_d \cong 90$ μM.

Thus, all three classes of barbiturates act consistent with the notion that they bind to a site which modulates ACh binding, possibly the receptor protein itself. (Supported by GM-15904 and GM-07592).

92.13 BLOCKADE OF GLUTAMATE-MEDIATED CHLORIDE CONDUCTANCES BY QUINUCLIDINYL BENZILATE IN APLYSIA NEURONS. M. Filbert* and D. Weinreich. Neurotoxicology and Experimental Therapeutics Branch, US Army Medical Research Institute of Chemical Defense, APG, MD 21010 and Department of Pharmacology and Experimental Therapeutics, University of Maryland, School of Medicine, Baltimore, MD 21201.

Quinuclidinyl benzilate (QNB), the benzilic acid ester of 3-quinuclidinol, has been used extensively as a specific probe for muscarinic acetylcholine (ACh) receptors in both the peripheral and central nervous system. Both QNB and atropine have been reported to interact with the nicotinic receptor-ionic channel complex of skeletal muscle (Schofield et al., 1981), yet Yamamura and Snyder (1974) report no detectable specific binding of QNB at rat diaphragm. These findings suggest that both atropine and QNB could have non-ACh related actions when applied to neurons possessing multiple neurotransmitter recognition sites, whether associated with similar or dissimilar ionic channels. Using current- and voltage-clamp techniques, the effects of QNB were examined on identified cerebral and buccal neurons of Aplysia. Agonists were applied by iontophoresis from double-barrel micropipettes. Antagonists were delivered by superfusion into a 0.5 ml recording chamber which was continually perfused (1-2 ml/min) with artificial seawater. QNB (10^{-4}M) totally abolished glutamate-mediated chloride conductance (GLU-Cl^-) while having relatively minor effects (10-20% changes) in the amplitude of ACh-, GABA- or histamine-mediated ionic currents. The actions of QNB on GLU-Cl^- was: (a) dose-dependent (I_{50} = 5 x 10^{-5}M); (b) occurred without measurable effects on resting membrane potential, input inpedance or action potential waveform; (c) not mimicked nor blocked by atropine (10^{-5}M); and (d) reversed by perfusion with drug-free seawater. These results show that QNB is not exclusively a cholinergic antagonist. Furthermore, QNB may be a useful probe to characterize glutamate receptors in Aplysia.

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93.1 MODULATION OF SYNTHESIS OF APLYSIA EGG-LAYING HORMONE BY AGENTS WHICH INFLUENCE CYCLIC NUCLEOTIDE LEVELS. Charles L. Bruehl and Robert W. Berry. Dept. Cell Biol. and Anat., Northwestern Univ. Medical School, Chicago, IL 60611

The neurosecretory bag cells of Aplysia provide an attractive experimental system for studying the regulation of neuropeptide synthesis by factors which evoke secretion. These cells produce a peptide egg-laying hormone, ELH, via a well-defined processing sequence. Brief synaptic input results in a bout of repetitive discharge which lasts about 1/2 hr and which leads to ELH secretion. Repetitive discharge can also be elicited by the putative transmitter dopamine and is associated with an increase in cAMP levels. We have previously shown that potassium-induced depolarization of the bag cells results in an immediate 30% elevation in the rate of ELH synthesis, and that this effect is due either to the receipt of presynaptic transmitter or to the secretion of ELH itself (Berry and Arch, 1981, Brain Res. 215: 115). Since an increase in cAMP levels is a normal consequence of bag cell activation, we performed the following series of experiments to determine if the enhanced synthesis of ELH might be mediated by cyclic nucleotides. Bag cell organs were exposed to dopamine, 8-substituted cAMP analogs, or the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) for 3 hr., after which the incorporation of ^{3}H-leucine into ELH was measured over a 2 hr. period. Experimental organs were compared with paired control organs from the same animal which had remained in artificial seawater. Dopamine at 0.2mM had no significant effect on ELH synthesis, but at 1mM, elevated synthesis by 28%. Cyclic AMP analogs, at 0.5mM, increased ELH synthesis by 21%. IBMX had no effect on synthesis at 0.1mM, but led to an increase of 61% at 1mM. The dose-related effect of IBMX on ELH synthesis was well correlated with its effect on cAMP levels, as it produced no significant elevation of cAMP at 0.1mM, whereas it increased cAMP by 550% at 1mM. These results are consistent with the hypothesis that the rate of ELH synthesis is modulated by the receipt of presynaptic transmitter via changes in cyclic nucleotide levels.
(Support: NIH NS 11519.)

93.2 **DIRECT MEASUREMENTS OF THE EFFECTS OF CYCLIC AMP, AND RELATED COMPOUNDS, ON MEMBRANE CONDUCTANCES, INTRACELLULAR CALCIUM AND pH IN GASTROPOD NEURONS.**

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We have previously reported that injections of cyclic AMP into identifiable *Archidoris* neurons resulted in a decrease in intracellular pH recorded using the dye arsenazo III *(Soc. for Neuroscience Abstr.* #107.7, 1980), as well as dose-dependent effects on cellular firing patterns *(ibid.* #165.7, 1981). We have now examined each of these results in greater detail using more direct measurements - i.e. pH microelectrodes and voltage clamp techniques. In addition we have used the indicator dye arsenazo III to probe for changes in intracellular free Ca^{2+} following cyclic AMP injections.

Pressure or iontophoretic injection of cyclic AMP into any one of the fourteen identified neurons in *Archidoris* routinely resulted in the following changes in cellular properties: (1) a dose-dependent, reversible increase in membrane sodium permeability; (2) long-lasting changes in voltage-dependent membrane conductances leading to sustained depolarization and sometimes to a slow-burst firing pattern; and (3) a dose-dependent, reversible intracellular acidification which outlasted the transient sodium influx and persisted in the absence of extracellular sodium. Having the phosphodiesterase inhibitor IBMX (10^{-3}M) in the bath potentiated these cyclic AMP-induced responses. No change was found in the resting level of internal free Ca^{2+} nor in the characteristics of calcium influx or regulation during electrical activity following injections of cyclic AMP.

Intracellular injections of cyclic GMP, dibutyryl-cyclic AMP and 8-benzylamino-cyclic AMP resulted in similar membrane conductance and pH changes, although the two analogs' effects were much less reversible than either cyclic AMP or GMP. In addition the pH change induced by cyclic GMP characteristically had a faster onset and time to peak compared to a cyclic AMP-induced response. Comparable injections of 5′AMP or ATP did not elicit these responses. Finally, injections of cyclic AMP into neurons of nine other gastropod species (including *Aplysia)* yielded similar membrane conductance changes indicating that these induced effects may be generalizable to species other than *Archidoris*. (Supported in part by PHS Grants NS-15186 and GMO-7143).

93.3 CYCLIC AMP CONTENT OF A CONE-DOMINANT RETINA: TIME-COURSE OF LIGHT REDUCTION AND DARK RECOVERY, IN VIVO. Debora B. Farber, Dennis W. Souza* and Richard N. Lolley. Jules Stein Eye Institute, UCLA School of Medicine, CA 90024, and V.A. Medical Center, Sepulveda, CA 91343.

The cone-dominant retina of the 13-line ground squirrel (96% cones and 4% rods) contains 8-fold higher levels of cAMP than of cGMP. Light reduces selectively the cAMP content of the dark-adapted retina; cGMP content remains unchanged.

In order to determine the time required to decrease cAMP content from dark- to light-adapted levels, animals were dark-adapted for 3 hr and subsequently exposed to laboratory illumination for 1, 3, 5, 10, 15, 30 or 60 minutes prior to sacrifice. The eyes were enucleated and the retinas dissected sequentially. The first retina was extracted with 0.1 N HCl within 1-2 min of sacrifice, and the second retina was extracted about 1-2 min later. The time which elapsed between sacrifice and tissue extraction in acid was recorded in order to assess whether ischemia influenced the cyclic nucleotide levels. No statistical difference in cyclic nucleotide levels was observed between the first and second retina from the same animal. The cAMP content of the dark-adapted retina (91.0 pmol/mg protein) was reduced by 15% after 1 min, 50% after 3 min and 58% after longer periods of illumination.

The time during which cAMP levels returned to dark-adapted values was investigated. Following three hours of exposure of the ground squirrels to laboratory illumination, they were dark-adapted for 30, 45, 60, 75, 108 or 120 minutes before sacrifice. All subsequent operations were carried out under darkroom conditions. The cAMP content of the light-adapted retina increased gradually and reached the dark levels by 108 minutes of dark adaptation.

Our results indicate that light modulates selectively the metabolism of cAMP in ground squirrel retina, and they imply that cAMP is involved in cellular processes that are apparently slower than the visual response of cone visual cells.

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93.4 Characterization and Regulation of Particulate Cyclic GMP-Stimulated Cyclic AMP Phosphodiesterase (PDE) Activity of Rat Brain. M.A. Oleshansky* and R. Schwartz* (Spon: L.M. Neckers). Dept. of Psychiatry, New York University Medical Center, New York, New York 10016.

The hydrolysis of cyclic AMP and cyclic GMP at high and low substrate concentrations (300 and 3uM) was measured (in the presence of EGTA) in broken cell preparations of several areas of rat brain. Cerebral cortex and corpus striatum have high PDE activities for either substrate as determined at 300 uM relative to cerebellum in homogenate, supernatant and crude particulate preparations. At 3uM cyclic AMP, cerebral cortex and cerebellum have low PDE activity relative to corpus striatum, while at 3uM cyclic GMP, cerebral cortex and corpus striatum have nearly equal PDE activities while cerebellum has much lower levels of enzyme activity. Cyclic GMP (3uM) stimulated the hydrolysis of cyclic AMP (3uM) several fold more in broken cell preparations of cerebral cortex than in similar preparations of corpus striatum or cerebellum. This effect was most marked in particulate preparations of cerebral cortex. We have further characterized the hydrolysis of cyclic AMP in highly washed and sonicated particulate preparations of cerebral cortex. EGTA (10-500uM) lowered enzyme activity assayed at 3uM, nearly 70%, had no effect on hydrolysis at the 300uM substrate concentration and raised cyclic AMP hydrolysis (3uM) in the presence of cyclic GMP (3uM) by 50%. Thus, cyclic GMP stimulated cyclic AMP hydrolysis (assayed at 30° for 10 min) 2-3 fold in the absence of EGTA and 10 fold in the presence of EGTA. Cyclic AMP PDE activity (3uM) was extremely heat labile, reaching maximal activity when assayed for 10 min at 20° (-EGTA) and 10°C (+EGTA) and decaying rapidly when assayed at higher temperatures. Cyclic AMP hydrolysis at 300uM was maximal at 37°C. Cyclic AMP hydrolysis in the presence of cyclic GMP reached maximal activity at 20°C (-EGTA) and 30°C (+EGTA). Cyclic GMP did not stimulate the hydrolysis of cyclic AMP when assayed at 0-4°C. It appears that the stimulation of cyclic AMP hydrolysis by cyclic GMP is highly dependent on the temperature at which the assay is run and the presence or absence of EGTA.

93.5 CYCLIC AMP LEVELS IN LAMINAE OF THE RAT OLFACTORY TUBERCLE. N.R. Krieger. Depts. of Pharmacology and Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The rat olfactory tubercle is a thin sheet of cortex located at the ventral surface of the forebrain. Its outer layers are rich in dopaminergic terminals and dopamine-sensitive adenylate cyclase. Cyclic AMP levels in those layers may be an index of dopaminergic activity there. As a preamble to the demonstration of this possibility, we have used decapitation as a simple experimental means to raise cyclic AMP levels in the rat olfactory tubercle.

Male Sprague Dawley rats (200 g) were sacrificed by decapitation or by focussed microwave irradiation (3 sec; 1300W). After decapitation the brain was rapidly removed, hemisected, frozen in powdered dry ice and placed in a cryostat at -15°C. The brain surface at the tubercle was trimmed to a pedestal of 2 mm on a side. Consecutive tangential sections (16 μm) were cut in a plane parallel to the ventral surface of the brain and pooled in groups of 12. Every 13th section was stained. Tissue in 0.5 ml volumes (2 mM tris maleate, pH 7.4; 1 mM isobutylmethylxanthine) was homogenized, boiled, and assayed for cyclic AMP and protein. When sacrifice was carried out by microwave irradiation, the brain was rapidly removed and the tubercles dissected free, homogenized and boiled as above.

Cyclic AMP levels as a function of depth varied over a greater than 4 fold range. In the outer plexiform layer (0-200 μ) the level is highest (49 pmoles/mg protein). In the layers that include pyramidal cell bodies (200-400 μ) the level is 30 pmoles/mg protein. And just deep to the polymorphic domain of the tubercle, levels are as low as 11 pmoles/mg protein. After microwave irradiation, which blocks the post decapitation rise in cyclic AMP, we observed levels in the rat olfactory tubercle of 4.0 pmole/mg protein.

Thus, in the outer plexiform the post decapitation level was increased to 12 times the level seen after microwave irradiation. The increases are most dramatic in the plexiform and pyramidal layers (0-400 μ), the domain of the pyramidal cell bodies and their dendrites. We suggest that these neurons are the site of accumulation of cyclic AMP. The contribution made by glial cells and neuronal processes to these increases remains to be determined. Supported by NIH grant MH 31820

93.6 **IN VIVO Stimulation of cyclic AMP Formation by Dopamine in Rat Olfactory Tubercle.**
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Limbic forebrain structures such as the olfactory tubercle recieve extensive dopaminergic innervation from the ventral tegmentum and the substantia nigra. Cells which lie post-synaptic to these terminals are rich in receptors linked to a dopamine-sensitive adenylate cyclase. Studies of this enzyme in homogenates of the rat olfactory tubercle have described its laminar distribution and sensitivity to antipsychotic drugs (Krieger, N.R., et al., Brain Research 131 : 303-312, 1977). In the present study we injected dopamine directly into the rat olfactory tubercle and observed an in vivo stimulation of cyclic AMP levels.

Anesthetized animals (Nembutal, 50 mg kg^{-1} i.p.) recieved an intraperitoneal injection of the cyclic nucleotide phosphodiesterase inhibitor, isobutylmethylxanthine (IBMX, 15 mg kg^{-1}). Two minutes later, animals recieved an injection of dopamine directly into the olfactory tubercle (0.1-200 micrograms dissolved in a final injection volume of 1 microliter of artificial cerebral spinal fluid). Seven minutes post-injection of dopamine, animals were sacrificed by focused microwave radiation and their brains removed. Following excision of the olfactory tubercle, the tissue was homogenized and assayed for cyclic AMP and protein content. Control animals were intraperitoneally injected with IBMX, intracranially injected with vehicle only (CSF) and sacrificed according to the same protocol as experimental animals.

Results showed a dose-dependent stimulation of cyclic AMP levels by dopamine with a maximum stimulation of greater than three times control values (control range = 3.5 - 6.5 pmol mg $protein^{-1}$). The stimulation by dopamine was blocked by trifluoperazine. This is the first report of an in vivo dopamine-stimulated increase in cyclic AMP levels. This system should prove useful for studying the actions of drugs used to treat psychoses and Parkinson's Disease.

This work was supported by NIH 31820.

93.7 SUSTAINED INCREASE IN BASAL CYCLIC AMP IN THE HIPPOCAMPUS AFTER A MEDIAL SEPTAL LESION IN THE RAT. Estrada Bernard and James N. Davis. Veterans Administration Medical Center and Departments of Medicine (Neurology) and Pharmacology, Duke University, Durham, NC 27710.

Sympathetic fibers appear in the rat hippocampal formation after lesions of the septal input to this region. In order to study the function of this sympathetic ingrowth, we measured the content of cyclic 3′,5′-adenosine monophosphate (cAMP) in both hippocampal formations of adult male rats. In some animals, cAMP levels were measured immediately after stimulating the preganglionic trunk of one superior cervical ganglion at ten times threshold for one minute. All animals were sacrificed with focused microwave irradiation.

Unexpectedly, cAMP levels were markedly increased (0.56 ± 0.04 pmol cAMP/mg wet wt.) in animals 4 weeks after septal lesions compared to sham-operated or normal controls (0.27 ± 0.03). This increase occurred over the first week after septal lesion reaching 0.53 ± 0.08 pmol/mg by seven days and remained elevated four weeks after lesion, the longest time period studied. cAMP levels were significantly increased by DMI pretreatment in both controls (0.45 ± 0.06, $p < 0.01$ vs. saline pretreatment) and septal lesioned animals (0.79 ± 0.06, $p < 0.001$ vs. saline, 4 weeks). Sympathetic stimulation did not significantly increase hippocampal cAMP levels compared to the contralateral unstimulated side in either normal or septal-lesioned (4 weeks) animals. cAMP levels in septal lesioned animals were not affected by superior cervical ganglionectomy or pretreatment with oxotremorine, while cAMP levels in controls were not altered by treatment with scopolamine.

Since cAMP has an established role in neuromodulation, the striking and long-lasting elevations in hippocampal cAMP content demonstrate a profound septal influence on hippocampal function. The increase in cAMP appears unrelated to sympathetic ingrowth or to the loss of muscarinic cholinergic influence. The fact that DMI pretreatment further increases cAMP levels in septal lesioned animals raises the possibility that central noradrenergic neurons are involved in evoking the increase. The increase in cAMP may be related to the increased spontaneous activity of CA3 pyramidal neurons observed in similar animals (Ropert, N. et al., this meeting). This is the first observation of a long-lasting change in the cAMP level of a brain region resulting from a remote lesion and could have important implications for the recovery of function after brain injury.

(Supported by NS 06233)

93.8 CORTICOSTERONE MODULATION OF NORADRENALINE STIMULATED ADENYLATE CYCLASE IN HIPPOCAMPUS. V.J. Roberts*, R.L. Singhal* and D.C.S. Roberts[1] (SPON: K.C. Marshall). Department of Pharmacology, University of Ottawa and [1]Department of Psychology, Carleton University, Ottawa.

There has been much research indicating that antidepressant treatment decreases the sensitivity of norepinephrine (NE) receptor-coupled adenylate cyclase systems in brain. It has been hypothesized that depression occurs as a result of an increase in the sensitivity of the cyclic AMP generating system and antidepressant therapy alters this supersensitivity. There is also evidence suggesting that adrenocortical steroids may have a role in affective disorders as steroid imbalances and steroid therapy are often accompanied by mood disorders. We were therefore prompted to investigate a possible interaction between adrenal corticoids and the NE-coupled cyclic AMP generating system. Male Wistar rats (300 g) underwent bilateral adrenalectomy one week prior to sacrifice. Control rats were sham-operated. The hippocampi from two rats were pooled, slices prepared which were then incubated in 0, 1, 10 or 100 μM NE for 10 min according to the method of Harden et al. (J. Pharm. Exp. Ther., 203:132, 1977). Adrenalectomy was found to increase significantly the rate of cyclic AMP formation. Implantation of corticosterone pellets at the time of surgery completely reversed this increase following adrenalectomy. This is in accord with the findings of Mobley and Sulser (Nature, 286:608, 1980) who reported a similar effect in the frontal cortex. Metopirone, which inhibits corticosterone synthesis, was also found to increase cyclic AMP formation. This elevation was observed 2 hours following a 50 mg/kg i.p. injection and was also reversed by corticosterone pellet implantation. Our results suggest that adrenal glucocorticoids may regulate adrenergic-mediated responses in brain and that this interaction may be a rapid modulatory process which responds to apparent physical and emotional stressors. (Supported by grants from the Ontario Mental Health Foundation to Dr. R.L. Singhal and the Medical Research Council to Dr. D.C.S. Roberts).

93.9 STIMULATION OF A HIGH AFFINITY GTPase ACTIVITY BY ACETYLCHOLINE IN RAT STRIATUM. P. Onali, M. Olianas*, J.P. Schwartz and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

The hydrolysis of GTP by specific GTPase(s) is a crucial step in the regulation of the adenylate cyclase activity (a.c.). This GTPase activity is stimulated by various hormones which also affect a.c. activity. Previous studies from this laboratory have shown that muscarinic agonists inhibit a.c. activity of synaptic plasma membranes (SPM) of rat striatum. This inhibition was dependent on the presence of GTP and was not detected when GMP-PNP, a less hydrolyzable analog of GTP, substituted for GTP. This observation led us to investigate the effect of acetylcholine (ACh) on the activity of GTPase(s) present in SPM of rat striatum. By monitoring the release of ^{32}P-phosphate from γ-^{32}P-GTP (Cassel and Selinger, Biochim. Biophys. Acta 452, 1976) under the same conditions as are used in the a.c. assay, we have observed that striatal SPM contain a "high affinity" GTPase activity which accounts for approx. 60% of the total phosphate released at a GTP concentration of 0.1 µM. Isotope dilution curves showed that this GTPase activity decreased with increasing concentrations of GTP over the range from 0.05 to 2 µM but reached a plateau at higher concentrations of the substrate. The app. Km of this enzyme for GTP was approx. 0.8 µM and the app. Vmax approx. 700 pmoles/min/mg prot. The hydrolysis of GTP by this enzyme activity was inhibited competitively by GMP-PNP with an app. Ki of 0.7 µM. When ACh (100 µM) was present in the reaction mixture, the activity of this GTPase was stimulated at concentrations of GTP ranging from 0.05 to 2 µM, but not at a concentration higher than 30 µM. The stimulatory effect of ACh was mainly due to an increased Vmax rather than to a change in the Km for GTP. The effect of ACh was concentration-dependent with an app. EC_{50} of approx. 3 µM, a value similar to that found for the ACh inhibition of striatal a.c. activity. The maximal activation of the "high affinity" GTPase occurred at approx. 10^{-4}M ACh and represented a 30-50% increase over the basal enzyme activity. Atropine (0.1 nM to 10 µM) failed to affect basal GTPase activity but antagonized the stimulatory effect of ACh in a dose-dependent manner. Nicotine (100 µM) did not change GTPase activity. These results indicate that occupancy of striatal muscarinic receptors is associated with an increased activity of a "low Km" GTPase. The stimulation of GTP hydrolysis may be involved in the mechanism of inhibition of adenylate cyclase activity by ACh.

93.10 ULTRASTRUCTURAL DISTRIBUTION OF THE CYCLIC GMP SYSTEM: A ROLE IN NEUROTRANSMISSION IN THE RAT STRIATUM. M.A. Ariano, Anatomy & Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

The subcellular distribution of guanylate cyclase (EC 4.6.1.2), cyclic 3',5'-guanosine monophosphate (cyclic GMP), cyclic GMP-dependent protein kinase (EC 2.7.1.38), and cyclic GMP phosphodiesterase (EC 3.1.4.17) have been examined under basal conditions in rat striatum. Immunofluorescent localization of guanylate cyclase, cyclic GMP, and cyclic GMP-dependent protein kinase demonstrates coincident reactivity for these compounds within the cytoplasm and processes of ovoid and rounded neurons, 15-20 µm in diameter. Nuclear staining was characteristically absent in the frozen-sectioned material. Transcardiac perfusion with aldehydes followed by PAP immunochemical localization demonstrated no obvious translocations of immunoreactivity for these three antigens. Ultrastructural examination of caudate-putamen tissue demonstrates an exclusively neuronal localization for the cyclic GMP system. Guanylate cyclase, its product cyclic GMP, and the cyclic GMP-dependent protein kinase are immunocytochemically distributed within the spiny-type I neuron population (DiFiglia, et al, Brain Res. 114: 245, 1976) of the rat striatum. A synaptic compartment is also reactive for these three antigens. The hydrolytic enzyme, cyclic GMP phosphodiesterase has been determined previously (Ariano & Appleman, Brain Res. 177: 301, 1979) to be primarily confined to the junctional postsynaptic density region of asymmetrical axospinous terminals. Its substrate, cyclic GMP is principally distributed within the postsynaptic dendroplasm of equivalent synaptic boutons (Ariano & Matus, J. Cell Biol. 91: 287, 1981). The protein kinase and guanylate cyclase are apparent at presynaptic and postsynaptic sites of asymmetrical synaptic terminals.

Radioimmunoassay and enzyme-linked immunoabsorbant assay analysis of these different antibodies has demonstrated their specificity and suggest that a portion of the cyclic GMP immunoreactive sites are due to immobilization of the cyclic nucleotide onto the protein kinase moiety. Specific immunochemical staining for each of the cyclic GMP system components could be eliminated by prior incubation of the antibody with their respective purified antigens.

The subcellular distribution of the cyclic GMP system, determined through immunochemical and enzyme cytochemical reactivity, at selective synaptic terminal areas within the caudate-putamen suggests an association with synaptic transmission.

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93.11 MICROTUBULE DISRUPTING AGENTS AND Ca^{2+}/CALMODULIN DISASSOCIATE THE REGULATORY SUBUNIT OF ADENYLATE CYCLASE FROM THE SYNAPTIC MEMBRANE. Mark M. Rasenick, Carolyn M. Kosack*, and Robert J. DeLorenzo. Dept. of Neurology, Yale Univ. School of Medicine, New Haven, CT 06510

Recent studies have demonstrated that two tubulin binding agents, colchicine (CL) and vinblastine (VB) alter the activation of adenylate cyclase (AC) in synaptic membrane, suggesting that cytoskeletal proteins might modulate AC activity (Nature 294: 560, 1981). Activation of rat cerebral cortex synaptic membrane AC by NaF or guanylylimidodiphosphate [Gpp(NH)p] was enhanced ($\sim$2x) by incubation of membranes with CL and VB. However when membranes were washed subsequent to incubation with CL or VB, a 50% loss in activation of AC by Gpp(NH)p or NaF was noted. Calmodulin (CM) and Ca^{2+} have been demonstrated to activate a tubulin kinase in brain membrane and cytosol (Fed. Proc. 41: 2265, 1982; Brain Res. 236: 393, 1982). To ascertain whether Ca^{2+}-CM stimulated phosphorylation of tubulin had a similar effect on AC as CL or VB, membranes were incubated with Ca^{2+}-CM under conditions promoting the phosphorylation of membrane tubulin. Under these conditions, NaF and Gpp(NH)p activation of AC are enhanced ($\sim$2x) without affecting AC activity in the presence of Mn^{++}. If synaptic membranes are washed subsequent to incubation with Ca^{2+}-CM, about 50% of AC activation in the presence of Gpp(NH)p or NaF is lost while AC activation by Mn^{++} is unaffected. These effects of Ca^{2+}-CM on AC are similar to those of CL and VB when assessed in the same experiments and Ca^{2+}-CM plus CL are not additive.

These data suggest that Ca^{2+}-CM, CL and VB act on the GTP binding regulatory subunit (G unit) of AC, as Mn^{++} activation of AC, which is independent of the G unit, remains constant. Association of the G unit with the synaptic plasma membrane appears altered by Ca^{2+}-CM, CL or VB. Material released from membranes treated with Ca^{2+}-CM, CL or VB is capable of reconstituting "G unit activity" to synaptic membranes depleted of G unit or to G unit deficient cyc^- membranes. Release of synaptic membrane G unit by Ca^{2+}-CM, CL or VB is also indicated by preferential elution of a 42K azido anilido GTP labelled protein from synaptic membranes.

It is possible that membrane associated tubulin serves as a modulator of AC activity or a site of attachment for the AC G unit to the synaptic plasma membrane. Alteration of membrane tubulin by Ca^{2+}-CM dependent phosphorylation or CL or VB binding might thus regulate AC activity or binding of the G unit to the membrane. These results are consistent with an hypothesis that cytoskeletal dynamics might regulate a variety of membrane associated enzymes.

93.PO SIMILAR EFFECTS ON BODY TEMPERATURE IN THE RABBIT INDUCED BY DIBUTYRYL CYCLIC AMP AND GMP. S.B.Kandasamy*and B.A. Williams* (SPON: R.A. Nichols). Biosystems Division, NASA-Ames Research Center, Moffett Field, CA 94035.

Intracerebroventricular (ICV) administration of dibutyryl (Db) cAMP (30-200 µg/Kg) and Db-cGMP (50-200 µg/Kg) induced hyperthermia in rabbits. The object of our study was to find out the effect of some phosphodiesterase inhibitors on Db-cAMP and Db-cGMP-induced hyperthermias and to elucidate the central transmitters and receptors involved in these hyperthermias. Unless otherwise mentioned 50 µg/Kg, ICV of Db-cAMP and Db-cGMP were used as a test dose throughout our experiments. Pretreatment of a prostaglandin synthesis inhibitor indomethacin (1-5 mg/Kg, SC) did not alter the hyperthermia caused by Db-cAMP and Db-cGMP. Theophylline (10-50 µg/Kg, ICV) accentuated the hyperthermia due to Db-cAMP and Db-cGMP. 10-30 µg/Kg, ICV of 4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone (ZK 62711), a selective inhibitor of cAMP phosphodiesterase only accentuated the hyperthermia due to Db-cAMP whereas a selective inhibitor of cGMP phosphodiesterase 2-O-propoxyphenyl-8-azapurin-6-one (M&B 22948) administered centrally (10-30 µg/Kg, ICV) only potentiated the hyperthermia caused by Db-cGMP. ICV administration of a protein synthesis inhibitor anisomycin (100-500 µg/Kg) did inhibit the hyperthermia due to Db-cAMP and Db-cGMP.Phenoxybenzamine (1-5 µg/Kg, ICV) and d-tubocurarine (1-3 µg/Kg, ICV) did not antagonize the hyperthermia induced by Db-cAMP and Db-cGMP. Central administration of naloxone (1-10 µg/Kg, ICV) caused a dose-related reduction in morphine, ß-endorphin and (D-ala)^2-methionine-enkephalinamide-induced hyperthermias but had no significant inhibitory effect on the hyperthermia due to Db-cAMP and Db-cGMP.However pretreatment with a ß-adrenergic receptor antagonist sotalol (50-200 µg/Kg, ICV) specifically attenuated Db-cAMP-induced hyperthermia while a cholinergic muscarinic receptor antagonist atropine (5-20 µg/Kg, ICV) only antagonized Db-cGMP-hyperthermia. These results indicate that a protein mediator may be implicated in the induction of hyperthermia by Db-cAMP and Db-cGMP and that cAMP and cGMP may be involved through ß-adrenergic and cholinergic muscarinic receptors respectively in the central regulation of heat production/conservation in rabbits.

94.1 DEVELOPMENT OF AUDITORY NERVE FIBER RESPONSES IN KITTENS. E. J. Walsh*, J. McGee* and E. Javel. Boys Town Institute and Dept. of Physiology, Creighton Univ. Sch. of Med., Omaha, NE 68131.

Responses of single auditory nerve fibers were recorded from anesthetized kittens 7-30 days of age. Stimuli were monaural pure tones. Frequency-threshold curves (FTCs) taken during the first 7 postnatal days (pnds) showed extraordinarily broad tuning and high (120-130 dB SPL) thresholds. Units were responsive only to frequencies below 5 kHz. Poststimulus time histograms (PSTHs) typically showed an onset peak followed by a period of "rhythmic" response. Some units, however, exhibited only sustained responses. Dynamic range, spontaneous discharge rate and maximum discharge rate were uniformly low, and discharge synchronization could not be maintained above 2 kHz. Some neurons were encountered that, although spontaneously active, could not be driven by tones presented at 140 dB SPL. By 11 pnds, responses to frequencies above 5 kHz could be obtained. These neurons were tuned, but thresholds at characteristic frequency (CF) were still elevated (approx. 90 dB SPL), and tip-to-tail ratios were only 20-30 dB. PSTHs exhibited only sustained responses. Dynamic range, maximum discharge rate and synchronization, while improved relative to earlier ages, still displayed immaturity. When they could be recorded, low-CF neurons showed grossly immature responses. High-CF FTCs were mature by the 16th pnd. Thresholds at CF were as low as 10 dB SPL, tuning was sharp, and tip-to-tail ratios were 40-50 dB or greater. Dynamic range, maximum discharge rate and synchronization behavior were adult-like by the end of the third postnatal week.

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94.2 EFFECTS OF CONTINUOUS BACKGROUND STIMULI ON RATE RESPONSE OF AUDITORY NERVE FIBERS IN ANESTHETIZED CAT. E. D. Young and J. A. Costalupes. Dept. of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

An interesting current problem in auditory neurophysiology is the means by which the auditory system attains its wide dynamic range in the face of the restricted dynamic range of peripheral auditory neurons. We have studied the response properties of auditory-nerve fibers to best frequency (BF) stimuli in the presence of broadband noise and BF-tone backgrounds. The backgrounds were on continuously so that long-term adaptive effects could be observed. The BF-tone test stimuli were presented either in the presence of the uninterrupted background (for noise backgrounds only) or during a brief interval in which the background was turned off (negatively gated). 200 ms test stimuli were presented once every second. Background sound levels ranged from 20 to 90 dB SPL.

Negatively-gated backgrounds affected units' discharge rates in response to test stimuli in two ways: first, there was a reduction of rate at all sound levels, and particularly a reduction in saturation rate; second, there was a horizontal shift of the dynamic part of units' rate versus level functions to higher levels. The horizontal shift has not been observed in studies of short-term adaptation. We have found that the effects of backgrounds can be summarized by expressing a unit's rate versus level function in the presence of the background ($r_b(i)$) as a shifted and scaled version of its rate level function in quiet ($r_q(i)$). That is,

$$r_b(i) = S \cdot \left[r_q(i - i_h) - sp \right] + T \cdot sp$$

where i is test stimulus level and sp is spontaneous rate. The scale factors S and T represent the reduction in the unit's driven and spontaneous rates, respectively. S decreases monotonically as background level increases. i_h represents the horizontal shift of the unit's dynamic range. Horizontal shift increases roughly linearly with background level for levels above a certain threshold. The rate of increase varies between 0.2 and 0.5 dB/dB. This horizontal shift extends dynamic ranges somewhat but is not sufficient to prevent high background levels from producing a saturated discharge rate.

The effects of uninterrupted noise backgrounds on test rate differ from those of negatively-gated backgrounds in that there is additional suppression of response to the test stimulus; the rate of increase of horizontal shift was 0.5-1.0 dB/dB. The slope increase over the negatively-gated case probably comes from two-tone suppression. Furthermore, at higher levels the background stimulus itself produces a saturated discharge.

94.3 ADAPTATION IN THE DORSAL COCHLEAR NUCLEUS OF THE UNANESTHETIZED, DECEREBRATE CAT. D. J. Gibson and E. D. Young. Dept. of Biomedical Engineering, Johns Hopkins U. Sch. of Med., Baltimore, MD 21205

Recent studies (see Young and Costalupes, this volume) have found that auditory nerve fibers have a limited ability to adjust their dynamic ranges (adapt) to background stimuli. Previous studies in anesthetized animals had suggested that cochlear nucleus units might adapt to a greater degree. We have studied the effects of background sounds on unit discharges in the decerebrate cat's dorsal cochlear nucleus. Units were classified according to the scheme of Young and Brownell (J. Neurophys. 39: 282, 1976) and Young and Voigt (Hearing Res. 6: 153, 1982), where type II units have low spontaneous rates and respond poorly to noise, type III units have spontaneous activity and inhibitory sidebands, and type IV units have spontaneous activity and highly nonmonotonic rate-level functions at best frequency (BF), with inhibitory responses at intensities more than 30 dB above threshold.

In order to determine the degree to which a unit adapted to a background, the unit's rate-level function was measured in the presence and in the absence of the background, using 200 ms test tones at BF. The background was either a BF tone presented continuously except during the test tone, or broad-band noise presented continuously and persisting during the test tone. Adaptation was defined as the shift of the rate-level function towards higher intensities produced by the background, and was measured by the method described by Young and Costalupes (this volume). The adaptation slope is given by (A2-A1)/(BG2-BG1), where A1 is the shift in dB produced by a background whose intensity was BG1, and A2 is the shift produced by BG2.

Units of all three types showed adaptation slopes that were comparable to those found in auditory nerve fibers. Tone backgrounds elicited adaptation with slopes generally in the 0.4 to 0.6 dB/dB range, while noise backgrounds produced adaptation slopes which were generally between 0.8 and 1.2. However, type II and IV units differed significantly from auditory nerve fibers in that the dynamic regions of their rate-level functions were preserved in the presence of continuous noise backgrounds to a greater degree than in the auditory nerve. Since little discharge was evoked in the type II units by noise backgrounds, their incremental responses to test tones remained substantial even at high levels of background noise. Type IV units responded to the background noise, but the inhibition evoked by BF test tones persisted, so that the inhibitory dynamic region of the rate-level function was preserved. Thus, the ability of type II and IV units to signal the intensity of the test tone relative to that of the noise background remained substantially unaltered over a wide range of background intensities.

94.4 DEVIATIONS FROM LINEARITY IN AUDITORY NERVE-FIBER RESPONSES TO SPEECH. Donal G. Sinex* Central Institute for the Deaf, St. Louis, MO 63110, and C. Daniel Geisler, University of Wisconsin, Department of Neurophysiology, Madison, WI 53706. (SPON: D. Eldredge).

Responses of auditory-nerve fibers to synthesized speech stimuli were obtained from anesthetized cats. The simplified consonant-vowel (CV) syllables included a transition, during which the frequencies and amplitudes of spectral peaks were changing fairly rapidly, and a steady-state /a/ vowel. The neural data were analyzed in terms of average discharge rates and in terms of discharge synchrony. Average discharge rates, calculated for successive 8-msec windows over the duration of each syllable, appear to provide more information about the spectra of CV syllables than they do about steady-state vowels. However, the short-term spectra of the syllables are represented in more detail by the timing of discharges. As determined by Fourier analysis of compound period histograms, the dominant frequencies in the fibers' discharge patterns are highly correlated with the frequencies of prominent peaks in the short-term spectra of the speech stimuli. Familiar non-linear effects, such as synchrony suppression, are evident in the discharge patterns and appear to enhance the representation of spectral peaks. However, the complexity of the stimulus, particularly during the transition portion of the syllable, makes a quantitative analysis of the magnitude of the non-linearity difficult. For our analysis, we compared each neuron's responses to predictions from a linear model. The model was derived from each fiber's tuning curve and has previously been found to provide a good account of VII-th nerve responses to clicks and brief tone bursts. Particularly at high SPL, the synchrony of neural discharges is dominated by lower-frequency stimulus components than is the response of the model. In addition, a given spectral peak may be more prominently represented in the neural data than is predicted by the linear model. This property of the neural response may aid in the extraction of important features of the CV syllables.

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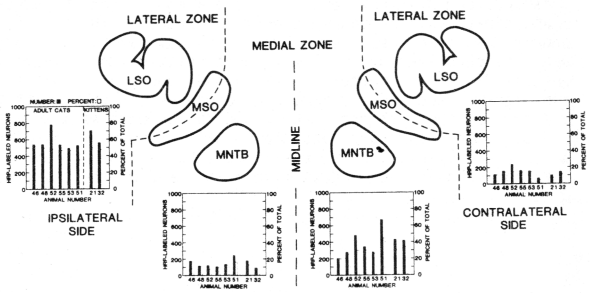
94.5 ENZYMES OF ACETYLCHOLINE METABOLISM IN CENTRIFUGAL FIBERS TO THE INNER EAR OF THE RAT. D.A. Godfrey[1], J.L. Park[1]*. J.D. Dunn[2] and C.D. Ross[1], Depts. Physiol.[1] and Anat.[2], Oral Roberts Univ., Tulsa, OK 74171

It has been known for many years that the centrifugal fiber bundles from the brainstem to the inner ear stain positively for acetylcholinesterase (AChE) activity. We have found that considerable lengths of these bundles can also be visualized in unstained, unfixed freeze-dried sections through the brainstem of the rat. Thus, once the bundles have been located in sections stained for AChE activity, pieces of them can be directly dissected at high resolution from adjacent freeze-dried sections, and quantitative measurements made of choline acetyltransferase (ChAT) or AChE activities. Data have been obtained for an AChE-positive bundle of fibers which fits the location of the olivocochlear pathway. Activities of ChAT for this bundle are comparable to those for the facial root dissected from the same brain, while activities of AChE are about twice as high as for facial root. (The facial root is taken as representative of a cholinergic nerve bundle since it contains the axons of only motoneurons.) Such a result is consistent with an interpretation that all axons in this (olivocochlear) bundle of fibers are cholinergic. In experiments in which this bundle was cut in the brainstem, the portion peripheral to the cut, as well as the central part, still stains for AChE activity after 2, 7 and 34 days survival, but the stain in the peripheral part has a granular rather than a smooth linear texture. The peripheral portion of the cut bundle can also still be recognized in the freeze-dried sections, although not as distinctly as in the case of the uncut bundle, and its ChAT activities were 2, 0.2 and 4% of control-side values in rats after 2, 7, and 34 day survivals, respectively, while AChE activities were 42% of control side values in the 7-day-survival rat. The ChAT results are consistent with a purely centrifugal direction of this fiber bundle, while the AChE results suggest that this enzyme activity is highly resistant to turnover in these axons when separated from their somata. When the cochlear nuclei were examined in rats sacrificed 7 days after unilateral transection of the olivocochlear bundles in the brainstem, ChAT activities in granular regions on the lesion side were about 60%, while activities in other parts were about 80% or more of control-side values. Such results suggest that most of the cholinergic synapses in the rat cochlear nucleus derive from sources other than the olivocochlear bundles. (Supported by ORU Intramural Funds and NIH Grant #NS17176.)

94.6 OLIVOCOCHLEAR NEURONS: QUANTITATIVE COMPARISON OF THE LATERAL AND MEDIAL EFFERENT SYSTEMS IN ADULT AND NEWBORN CATS. W.B. Warr, J.S. White and M.J. Nyffeler*. The Boys Town Institute for Communication Disorders in Children, Omaha, NE 68131.

Our purpose was to determine if the number and distribution of olivocochlear neurons (OCN) in the adult cat differ from those in the newborn, an age at which the cochlea is both morphologically and functionally immature. Because recent evidence suggests that the superior olivary complex contains two distinct systems of OCN, lateral and medial, which project differentially to certain targets in the cochlea, we analyzed our data according to this potentially functionally-significant dichotomy. Anesthetized cats received injections of 20µl of 20% HRP-2% DMSO into the left scala tympani. After 1-3 days, brains were fixed by perfusion with an aldehyde mixture, and frozen sections processed for HRP demonstration using tetramethylbenzidine (adults) or o-dianisidine (kittens). Alternate sections were stained for AChE. The results revealed no obvious differences between the 6 adults and 2 newborn animals used in this study. The Fig. suggests that no consistent



differences exist in the distribution of OCN, expressed either as absolute number (left axes) or as percent of total (right axes). Interestingly, one cat (51) was exceptional in having, in toto, more medial than lateral zone OCN, and a correspondingly larger population of medial than lateral AChE-stained neurons. These data, although suggesting that the olivocochlear bundle of the cat, like that in chinchilla, is quite variable in composition (Iurato et al., J. Comp. Neurol., 182, 1978), nevertheless provide information necessary for estimating innervation ratios between lateral and medial OCN and their principal synaptic targets, the dendrites of spiral ganglion cells and the outer hair cells, respectively. (Supported by NIH-NS #14832).

94.7 DIFFERENTIAL DISTRIBUTION OF AFFERENT FIBERS IN THE DORSAL COCHLEAR NUCLEUS. F.H. Willard, R.H. Ho and G.F. Martin. Dept. of Anat., Coll. of Med., Ohio State Univ., Columbus, OH 43210.

The mammalian dorsal cochlear nucleus (DCN) is characterized by a row of principal neurons whose elongated dendritic domains traverse four cytologically distinct neuropil layers. These layers receive a differential complement of afferent projections in the tree shrew (Jones and Casseday, '79), suggesting a segregation of input along the dendrites of principal cells. We have further investigated the stratification of afferent fibers in the DCN using the methods of anterograde transport, axonal degeneration, and immunohistochemical techniques. We have initiated our studies on the North American opossum since its protracted period of neurogenesis in an external pouch offers opportunities for examining the role of afferent input on neuronal differentiation.

Lesions of the auditory nerve result in degeneration of primary afferent fibers and terminals throughout layers III and IV; only a few degenerating figures are seen in layer II. In layer III, the primary afferent fibers course parallel to the principal cell basal dendrites. ^{3}H-leucine injections of the superior olivary complex labelled fibers in the dorsal acoustic stria (DAS) which could be followed into layer III. Only a few labelled axons extended into layer II. Clusters of presumed terminals are present in layer III. Fibers containing ENK-like immunoreactivity are seen in the DAS, in the extreme margins of layer III and throughout layers II and I. Most ENK immunoreactive fibers in layer I display varicosities and are oriented perpendicular to the pial surface. That is, they course in the direction of the principal cell apical dendrites. Fibers containing 5HT immunoreactivity are present throughout layers I, III and IV, and to a slight extent in layer II; however, two different orientations are seen. 5HT immunoreactive fibers in layers I and IV are aligned parallel to the pial surface, whereas those in layer III are oriented perpendicular to this surface. In all regions, 5HT immunoreactive elements appear as beaded fibers. Control sera consisting of the various antibodies pretreated with the appropriate antigen failed to reveal any of the aforementioned immunoreactivities on adjacent sections.

In summary, a differential distribution of input to the opossum DCN exists; auditory nerve and superior olivary complex afferent fibers are concentrated in layers III and IV, ENK immunoreactive fibers are present in layers I and II, and 5HT immunoreactive fibers innervate primarily layers I, III and IV. These results suggest that the principal cell dendritic domains, many of which span all four layers, are exposed to a stratified complement of afferent fibers. (Supported by BNS-80-08675 to G.F. Martin, NIH NS-10165 to R.H. Ho. We thank Dr. Robert Elde for the 5HT antibody).

94.8 TRANSGANGLIONIC TRANSPORT OF D-[^{3}H]-ASPARTATE (D-ASP) FROM COCHLEAR NUCLEUS TO COCHLEA IN THE CAT. D.R. JONES*, D.L. OLIVER, S.J. POTASHNER, D.K. MOREST. Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032

The connections of the outer hair cells and type II ganglion cells with the CNS and the identity of their transmitters are unclear at present. Previous reports suggested that both type I and II ganglion cells project to the cochlear nucleus and may use glutamate or aspartate as a transmitter (Anat.Rec.199:186A,1981; Abst. Assoc. Res. Otolaryng. 5:90,1982). 3 mM D-ASP, a putative marker for glutamatergic neurons, was injected into the cochlear nucleus of 3 cats, and the cochleas prepared for light microscopic autoradiography after survivals of 20 min, 5,and 48 hrs. Grain counts per unit area were made for each of 14 tissue compartments and normalized to permit comparisons between cases. After 20 min only the cochlear nerve and spiral limbus are labeled. A declining gradient of grains from the central end of the nerve indicates an incipient axonal transport, unrelated to the label in the spiral limbus seen in all 3 cases. After 5 hrs heavy labeling appears over cochlear nerve fibers, type I and II ganglion cell bodies,and beneath and around inner and outer hair cells in those compartments containing sensory fibers and endings. After 48 hrs the labeling pattern is unchanged, with two main exceptions: 1) the Schwann cell and satellite cell bodies are labeled and contribute to increased grain counts in the ganglion cell compartment; 2) grain counts in the organ of Corti have decreased to low levels.

The findings are consistent with uptake of D-ASP and retrograde transport by cochlear nerve axons from the cochlear nucleus to the perikarya and peripheral processes of the spiral ganglion. This interpretation is supported by the spatial and temporal gradients of labeling which appear in the cochlear nerve and subsequently in the spiral ganglion cell bodies and their peripheral processes. Labeling of these structures by an extra-neuronal route is unlikely since non-neuronal structures were unlabeled or only lightly labeled, except for Schwann cells and satellite cells in the ganglion after 48 hrs. However, these latter cells were unlabeled after 5 hrs when the ganglion cells and nerve fibers were heavily labeled. Evidently, D-ASP did not reach the ganglion cells and their peripheral endings via extra-neuronal pathways.

Thus, the available results show that axons of both type I and II ganglion cells project to the cochlear nucleus and that this nucleus is directly connected with both the inner and outer hair cells. Transganglionic transport of D-ASP from the cochlear nucleus is consistent with the hypothesis that the cochlear nerve axons use glutamate or aspartate as a transmitter.
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94.9 2-DEOXYGLUCOSE (2-DG) MAPPING OF GUINEA PIG AUDITORY NUCLEI AFTER SALICYLATE ADMINISTRATION. J.W. Nemitz, C.T. Sasaki*, J.S. Kauer. Sections of Neurosurgery, Neuroanatomy, and Oto-Rhino-Laryngology. Yale University School of Medicine, New Haven, CT 06510.

It is well known that tinnitus may be reversibly induced in humans by administration of high doses of salicylates (aspirin). In the present experiments we have examined changes in auditory neural activity using the 2-DG method after administration of salicylates in a guinea pig model for the study of tinnitus. Our hypothesis is that tinnitus results from aberrant increases in neural activity in the auditory pathway and that such increases may be observed by using 2-DG to measure changes in glucose uptake. To enhance possible aberrant increases in neural activity which might occur with salicylate administration, the experiments were carried out on animals in which basal activity levels were lowered on one side by removing the fused ossicle, thus causing a unilateral conductive hearing loss.

In 12 ketamine anesthetized guinea pigs (250-450 gms) the fused ossicles were unilaterally removed under sterile conditions without damage to the cochlea or tympanic membrane. A femoral catheter was inserted for assaying salicylate blood levels and for 2-DG administration. The day after surgery, the animals were force fed sodium (Na) salicylate (52 mg/100 gms body wt) in water. As peak blood concentrations of salicylate were reached, 2-DG was administered and the animals were divided into two groups: the first group was placed in a relatively quiet environment (65 dbC) for the 45 minute 2-DG test period, and the second group was placed in a white noise environment at 75 dbC. The controls consisted of animals which had unilateral ossicle removals, but no salicylate administration.

In the experimental animals given Na salicylate for two consecutive days, there were bilaterally elevated 2-DG levels in the dorsal cochlear nuclei, the nuclei of the trapezoid body, and the inferior colliculi compared to the control animals. Elevation of 2-DG uptake was particularly marked in the structures with predominant input from the lesioned side. The enhanced 2-DG uptake was slightly greater in those animals tested in the quieter environment. The pattern of uptake in the inferior colliculi of salicylate-treated animals consisted of bands aligned parallel to the tonotopic organization of the nucleus.

These results suggest that Na salicylate administration at levels which produce tinnitus in humans can cause increases in glucose uptake in auditory nuclei which have had their afferent input reduced by ossicle removal as well as in those nuclei receiving normal stimulation. We suggest that these increases in metabolic rate represent the presence of aberrant neural activity which may be a manifestation of a tinnitus-like phenomenon in this experimental animal. Supported by grant #NS-16288-01.

94.10 AN ANALYSIS OF HRP STAINED CELLS IN THE VENTRAL COCHLEAR NUCLEUS OF THE CAT. W.S. Rhode* and P.H. Smith. Dept. of Neurophysiology Univ. of Wisconsin, Madison, Wis. 53706.

We have made intracellular injections of the dye, horseradish peroxidase (HRP), into cells in the ventral cochlear nucleus displaying either a chopper or a primary-like response pattern to a short tone burst at the cell's characteristic frequency (STCF), and have subsequently analyzed the morphological characteristics of these cells at the light and electron microscopic level.

Those injected cells displaying the chopper pattern (a multimodal response, to STCF, whose mode is unrelated to stimulus frequency) are stellate cells. One such injected cell has a smooth cell body with few appendages. The three smooth primary dendrites radiate from the cell body, branching infrequently and giving rise to an occasional string-like appendage or a rare, more complex appendage. The axon arises from a primary dendrite, very close to its origin, and courses toward the trapezoid body giving off a recurrent collateral whose beaded branches ramify extensively in the cell's immediate vicinity. At the electron microscope level, few synapses were found on the cell body while an extensive number were seen on the dendrites. Some terminals of the recurrent collateral, which arises from the main myelinated axon, appear to synapse on large dendrites of neighboring cells. Many of the cell's features are comparable to the few stellate cells described by Tolbert and Morest (Neuroscience, In Press) which were backfilled when HRP was injected into the median nucleus of the trapezoid body and medial superior olive.

We have also injected cells wtih primary-like responses. As we reported previously, (Rhode, Oertel, and Smith, J. Neurosci., In Press) intracellular records reveal large, rapid depolarizations of variable size which appear to be synaptic potentials. Morphologically, these cells are bushy cells in the anteroventral cochlear nucleus. Cells collected thus far have smooth, rounded cell bodies with one or two smooth primary dendrites which arboralize extensively a short distance from the soma in the typical bush-like manner. The axon arises from the cell body and courses toward the trapezoid body without branching. The electron microscopic analysis of these cells will be discussed.

This work was supported by funds from NIH grant NS12732 and NS07026.

94.11 SPECIALIZED RESPONSES TO AMPLITUDE MODULATION IN THE POSTEROVENTRAL COCHLEAR NUCLEUS: SINGLE UNIT RECORDINGS WITH HRP-FILLED MICROPIPETTES. R. D. Frisina, R. L. Smith*, and S. C. Chamberlain*. Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

A new surgical approach has been developed for recording from single units in the cochlear nucleus (CN) of the anesthetized gerbil. It allows stable extracellular recordings from all the divisions of the CN (Frisina et al., Hearing Res. 6, 259-275, 1982), and stable intracellular and quasi-intracellular recordings for periods of up to 30 min or more.

We have used the new surgical approach to study single units that may be functionally specialized for encoding changes in sound amplitude (Møller, Acta Physiol. Scand. 86, 223-238, 1972). Stimuli consisted of 100-msec tone bursts at a unit's characteristic frequency. During the last 50 msec of the bursts, the average intensity was incremented by 6 dB and, in addition, the amplitude was sinusoidally modulated at a frequency of 150 Hz. Based upon extracellular responses to the amplitude modulated (AM) portion of the stimulus, single units were classified into two groups. One group showed a phase-locked response to the AM signal over a wide intensity range (threshold to 50-85 dB above threshold). The other group showed a phase-locked response over only a limited intensity range (threshold to 20-30 dB above threshold). Generator potentials showed two main components: an AC component at the frequency of amplitude modulation and a graded DC depolarization.

Additional extracellular response properties and location within the CN were then correlated with this initial classification. Units in the wide range group showed a CHOPPER response during the first 50 msec of the tone burst. Units in the limited range group showed either a CHOPPER or ON response. These particular ON units showed some residual firing during the duration of the tone unlike the ON units found in the octopus cell area of the PVCN. Onset and steady-state rate-intensity functions had essentially the same shape for both groups of units and saturated within 20-30 dB of threshold. The shape of the onset and steady-state rate-intensity functions may be related to the response to amplitude modulation for the limited range units. However, such a relationship cannot explain the response of the wide range units since they respond to amplitude modulation even where their rate-intensity functions have saturated. All the units of the present study were localized in the PVCN based on an iontophoretic injection of HRP. The results of this investigation may aid in understanding mechanisms of the mammalian auditory system for encoding changes in stimulus amplitude. [Supported by NSF and NIH.]

94.12 SOME EFFERENT PROJECTIONS OF THE DORSOMEDIAL PERIOLIVARY REGION. K. M. Spangler* (Spon: W. K. O'Steen) and C. K. Henkel, Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

The dorsomedial region surrounding the superior olivary complex, including the dorsomedial periolivary nucleus (DMPO) and the adjacent reticular formation, previously has been shown to project to the cochlea, to part of the ventral cochlear nucleus and to the midbrain tectum. Those cells which project to the cochlea via the olivocochlear bundle give a positive reaction for acetylcholinesterase (AChE). Using histochemical techniques we have undertaken a further investigation of the projections of this region and specific cell types involved, using the method of retrograde transport of HRP.

Cochlear injections were made in five adult cats. Injections involving the superior and/or the inferior colliculus were done in ten cats. Additional injections were made in the cervical cord and the facial motor nucleus. In most cases, alternate sections were reacted for the demonstration of AChE in addition to HRP. Following cochlear injections, cells that were positive for both HRP and AChE were seen in DMPO and appeared to correspond to the radiate neuron. There were also numerous AChE positive cells containing no HRP reaction product, probably indicating inadequate filling of the cochlea with HRP. No other apparent cell type in DMPO backfilled from the cochlea. Midbrain injections backfilled cells in the entire dorsomedial region. Cells were seen in DMPO, amid the rootlets of the sixth nerve, in the reticular formation adjacent to DMPO and lying within the dorsal part of the trapezoid body. Both the elongate and radiate neurons of DMPO appeared to backfill, but no cells were double labeled with AChE and HRP. Other cell types included large and small neurons in the reticular formation, a very small fusiform cell intercalated between the fibers of trapezoid body and an elongated cell that bordered and was parallel to the rootlets of the sixth nerve. After injections in the cervical cord, the large and small cells of the reticular formation were labeled; whereas, following injections in the facial nucleus and caudal superior olivary complex, cells in DMPO and cells surrounding the sixth nerve rootlets were backfilled. With such a wide variety of efferent projections, the dorsomedial periolivary region may prove to be an important area for complex interactions of centrifugal and auditory-motor channels.

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94.13 ULTRASTRUCTURAL ORGANIZATION OF THE LAMINAR NUCLEUS IN THE AUDITORY MIDBRAIN OF THE RED-EARED TURTLE. A.B. DRAKONTIDES AND R.H. BROWNER. DEPT. OF ANATOMY, NEW YORK MEDICAL COLLEGE, VALHALLA, N.Y. 10595.

The laminar (external) nucleus (LN) in the red-eared turtle is a prominent structure composed of 3 to 5 layers of mostly spherical neurons. It surrounds the central nucleus dorsomedially, medially and ventromedially throughout the rostrocaudal length of the torus semicircularis. The organization and light microscopic analysis of this nucleus has been previously reported (Browner et al., J. Morph., 169: 207-233, 1981).

The present study has been undertaken to further characterize the morphology of this area at the ultrastructural level. Analysis was limited to the coronal plane of section and to the mid- and rostral portion of the laminar nucleus. The following preliminary findings have been compiled. The organization of spherical somata within the layers of the laminar nucleus varied, and have been classified into two separate populations. One category contained clusters of 4 to 5 cells. Twelve to twenty clustered somata were observed extending in a linear fashion within the plane of section. Clustered cells occurred at 4.5 to 6.0 μm intervals. Intervals between clustered somata contained axonal and dendritic profiles. Few synaptic contacts were present on the free surfaces of clustered somata. Within a cluster, the cell membranes were closely apposed. At these soma-soma contacts, gap junctions were evident. In this population of neurons nuclei exhibited both condensed and extended chromatin. The cytoplasm contained typical organelles in addition to lysosomes and multivesicular bodies.

The second neuronal group of spherical perikarya were also closely apposed; however, the sites of apposition contained numerous lamellae. Their origin remained unresolved. The nuclei of these cells were vesiculated. An extensive number of lysosomes were present and the Golgi regions were more frequent and elaborate. In contrast to the paucity of synaptic contacts in the first neuronal group, these neurons had relatively more synaptic contacts.

The neuropil of the LN was similar to that seen in other amniotes. Axonal ending contained the following type of synaptic vesicles: a) both clear core (50 nm) and larger dense core (150 nm) vesicles; b) clear core vesicles; c) clear core vesicles and clumps of glycogen-like granules. Typical dendritic terminals were present. Numerous tracts that contained 20-50 unmyelinated axons with an average diameter of 230 nm were observed. There were few myelinated axons. This research was supported by the Whitehall Foundation.

94.14 Characteristic delays in the cat inferior colliculus. Yin, T.C.T. and Kuwada, S.*, Dept. of Neurophysiol., Univ. of Wisconsin, Madison, Wis. 53706

In 1966 Rose and his colleagues studied the response of low frequency cells in the cat inferior colliculus (IC) to changes in the time of arrival of tones to the two ears and found that the discharge rate was a cyclic function of interaural delay. When stimulated at different frequencies, the periodic interaural delay curves reached the same relative amplitude at the "characteristic delay" (CD) of the cell. Similar cells have been described in other auditory structures, but the evidence supporting CD does not really have strong experimental support since very few cells have been studied in detail, most studies have ignored the possibility originally suggested by Rose et al. (1966) that cells may show a CD at some point other than the peak or trough, and objective criteria for determination of CD have not been established. We have re-examined the issue of CD in an attempt to resolve these problems.

We recorded single cell activity from the IC of barbiturate anesthetized cats. Since the results obtained from the interaural delay and binaural beat stimuli are similar, we combined the results obtained from these two methods. For each cell the interaural phase sensitivity and mean interaural phase were determined at a number of frequencies. We then plotted the mean interaural phase vs frequency and determined with a statistical criterion whether it satisfied a linear relationship. For a linear phase-frequency function, the slope of the line is the CD and the phase-intercept is the characteristic phase (CP) of the cell. Neurons with a CD on the peak of the delay curves have a CP of 0.0, those with a CD on the trough have a CP of 0.5, and the value of the CD specifies the interaural delay of the common point. When the CD occurred at or near the peak or trough of the cyclic discharge functions, the results of this analysis procedure were generally in accord with the traditional method using visual inspection of the delay curves when plotted on a common time axis. However, in many cases visual determination of the CD was ambiguous and in such cases our analysis technique usually indicated a CD at some CP other than the peak or trough. Our sample of IC cells have a distribution of CD's such that about 75% lie between ±300 μsec while the CP's vary on a continuum between 0.0 and 1.0. Most (85%) have a CP which is not within 0.05 of 0 or 0.5, i.e., its CD is not at the peak or trough. Thus, our results support the original concept of a CD: cells may show a CD at any point along the cyclic interaural delay curves. However, some IC cells did not exhibit CD.

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94.15 Topographical relationships along the isofrequency laminae of the cat inferior colliculus: correlation with the anatomical lamination and representation of binaural response properties. Chan, J.C. and Yin, T.C.T., Dept. of Neurophysiol., University of Wisconsin, Madison, Wis. 53706

The central nucleus of the inferior colliculus (ICC), like most other major auditory nuclei, is tonotopically organized and receives binaural input. From dorsal to ventral cells have successively higher best frequencies and they appear to be organized in roughly parallel sheets. It is commonly assumed that these isofrequency laminae correspond to the layered arrangement of cells and their dendritic arbors which have been reported in Golgi studies, but this has not been verified directly. In this study we were interested in two questions. First, what is the relationship between the isofrequency laminae identified by physiological means and the dendritic laminae seen in anatomical preparations? Secondly, what stimulus parameters, if any, are represented along the two-dimensional isofrequency laminae, e.g., are cells in the same layer related to each other along some binaural cue? We have explored only the low frequency zone of the ICC with special emphasis on binaural interactions which involve interaural time differences.

To answer the first question, we made microelectrode penetrations from several different directions along the course of the isofrequency contours in the ICC of the anesthetized cat. We determined the best frequency of each cell encountered along the penetration. After physiological recording, the animal was sacrificed and the tissue was processed by the Golgi method in order to compare the course of the electrode track with the anatomical lamination. Our results indicate that the isofrequency laminae appear to match the layers established by the dendritic orientation of the disc shaped cells, at least in the low frequency zone of the ICC. To answer the second question, we studied the binaural response properties of each cell recorded along the isofrequency laminae with particular attention to cells which displayed characteristic delay (CD) using the method described by Yin and Kuwada (this volume). Our preliminary results indicate that there are iso-CD contours which are U-shaped with the open side of the U facing anteriorly. Cells around the periphery of the ICC tend to exhibit negative values of CD (corresponding to a longer transmission delay from the ipsilateral ear than from the contralateral) while those in the central regions show more positive CD's. We have also examined the distribution of characteristic phase and other binaural parameters along the isofrequency laminae.

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94.16 SINGLE-UNIT RESPONSES IN RAT INFERIOR COLLICULUS DURING IONTOPHORESES OF CHOLINERGIC AGENTS. G.R. Farley, B.J. Morley, E. Javel and M.P. Gorga*. Boys Town Institute for Communication Disorders in Children, Omaha, NE 68131.

Cholinergic agents were injected iontophoretically during unit recordings from the inferior colliculi of rats anesthetized with urethane. Carbamylcholine, a cholinergic agonist, increased tone-evoked activity in some units and decreased activity in others. Both muscarinic and nicotinic antagonists were also capable of influencing activity. A number of these antagonists, including atropine and curare, altered activity when injected alone, typically in an excitatory manner. Other antagonists, such as α-bungarotoxin, had little effect when delivered alone, but could block the effects of carbamylcholine. For response-intensity functions at unit characteristic frequency, the major effect of delivering cholinergic agents was to change maximal firing rate; threshold and dynamic range were generally unaltered. Applying a cholinergic agent typically altered activity across the entire response area of a unit, rather than in a specific frequency band. When the agent was excitatory, some broadening of the response area could be seen; when it was inhibitory, the response area was narrowed. These results suggest the presence of a cholinergically-mediated synaptic influence on inferior collicular neurons which modulates ongoing acoustic processing. [Work supported by NINCDS grant NS-14880 and NSF grant BNS-8006643.]

94.17 DESCENDING EFFERENT PROJECTIONS OF THE INFERIOR COLLICULUS: AN AUTORADIOGRAPHIC STUDY. D.I. Lugo* and M. H. Cooper, Departments of Anatomy and Otolaryngology, St. Louis University School of Medicine, St. Louis, MO 63104.

The efferent projection systems of the centrolateral and dorsal regions of the inferior colliculus (IC) were investigated in the laboratory rat (Rattus norvegicus albinus) using autoradiography. Tritiated leucine (0.05µl to 0.2µl) at a concentration of 50µCi/ul was injected stereotaxically into the centrolateral and dorsal regions of the IC of rats weighing between 290-330 grams with a Hamilton microsyringe equipped with a 26 gauge needle. Following a survival time of five days, the rats were perfused through the left ventricle and the brains were removed and processed for autoradiography according to the technique of Cowan et. al. (1972). The sections stained with cresyl violet were studied under bright field illumination for cytoarchitecture and nuclear arrangement and under dark field illumination for the course and terminations of the descending efferent projections.

Axons from the centrolateral region of the IC project to a variety of structures at the level of the IC as well in the caudal brainstem. At the level of the IC, bilateral terminal fields were observed in the cuneiform nucleus (CuN). Fibers travelled to the contralateral side through the commissure of the IC and areas of termination were observed in the contralateral central nucleus of the IC (CNIC) and external nucleus of the IC (ENIC). Caudally, axons streaming ventro-caudally from the IC traversed the medial and lateral parts of the lateral lemniscus (LL). Terminal fields were observed bilaterally in the paralemniscal nucleus (PL); reticular formation medial to the dorsal nucleus of the LL; superior olivary complex (SOC); and cochlear nuclei (CN). Ipsilaterally areas of termination were observed in the dorsal nucleus of the LL (DNLL), the reticular formation medial to the ventral nucleus of the LL and in the lateral pontine nucleus (LPN). Axons reached the CN by way of the trapezoid body.

The efferent fibers of the dorsal region of the IC gave a somewhat different pattern of termination than did those of the centrolateral region of the IC. At the level of the IC terminal fields were observed in the CuN bilaterally in the CNIC and ENIC contralaterally. Axons from the dorsal region of the IC travelled ventrally and caudally through the lateral part of the LL. Terminal fields were observed bilaterally in the PL and CN and ipsilaterally in the reticular formation medial to DNLL, LPN and SOC.

94.18 THE INFERIOR COLLICULUS PROJECTS TOPOGRAPHICALLY TO THE OPTIC TECTUM IN THE BARN OWL. E. I. Knudsen and. P. F. Knudsen*. (SPON: E. I. Knudsen). Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The optic tectum of the barn owl contains coincident maps of auditory and visual space. The auditory map is defined by a systematic change across the tectum in the locations of the auditory receptive fields of single neurons. Where does this space-specific auditory input come from? We found that the major source of auditory input to the optic tectum is a point-to-point projection from the space-mapped region of the inferior colliculus homologue, the MLD (mesencephalicus lateralis dorsalis).

Horseradish peroxidase (HRP) was injected iontophoretically into different portions of the optic tectum. After a survival time of 2 days the brains were perfused, and the tissue was sectioned and reacted for HRP using tetramethyl benzidine.

Each injection site in the optic tectum resulted in a single, dense cluster of labeled neurons located along the lateral or anterior border of the ipsilateral MLD. Neurons in this region have been previously shown to be space-selective, and to be organized according to their field locations into a map of space. No labeled neurons were found in the main (tonotopic) region of the MLD, nor in the contralateral MLD.

The location of the labeled neurons varied systematically with the site of injection in the tectum. Thus the MLD projection to the optic tectum is topographic: anterior MLD to anterior tectum, posterior MLD to posterior tectum, dorsal MLD to dorsomedial tectum, and ventral MLD to ventromedial tectum.

These data indicate that the map of auditory space in the optic tectum results from a point-to-point projection from specialized auditory neurons in the space-mapped region of the MLD. Based on the projection of the space-mapped region to the optic tectum, the position of this region in the MLD, and the binaural sensitivity and broad frequency-tuning of the neurons, the space-mapped region of the MLD appears to be analogous to the external nucleus of the mammalian inferior colliculus.

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94.19 FREQUENCY REPRESENTATION IN THE CAT'S MEDIAL GENICULATE BODY (MGB) AND POSTERIOR COMPLEX (PO). A. Morel* and T. J. Imig. Dept. of Physiology, Kansas Univ. Medical Ctr., Kansas City, KS 66103.

The spatial distribution of neurons' best frequencies in the MGB and PO was studied in pentobarbital anesthetized cats. Prior to the thalamic recordings, horseradish peroxidase (HRP) was injected into a physiologically defined portion of the cortical best frequency map. Neurons' responses to tone bursts were then recorded at 0.1 mm intervals along electrode penetrations passing through the thalamus either from dorsal to ventral or from dorso-caudal to rostroventral. Best-frequency maps in register with the pattern of thalamo-cortical projections were obtained by reconstructing electrode penetrations in tissue sections processed for HRP and counterstained with thionin.

Two complete tonotopic representations were found in portions of the MGB which correspond to pars lateralis (LV) and pars ovoidea (OV) as described by Morest (Morest, D.K., J. Anat., 98:611-630, 1964). Electrode penetrations passing through LV generally encounter orderly sequences of best frequencies. The low-to-high best-frequency gradient runs from lateral, caudal and ventral to medial, rostral and dorsal within LV. Within OV, the low-to-high best-frequency gradient runs from lateral to medial. The spatial distribution of best frequencies in OV exhibits somewhat more scatter than that in LV. LV and OV appear to adjoin along their low-to mid-frequency representations, although their high-frequency representations are discontinuous. Within the low and mid-frequency representations, isofrequency contours can often be reconstructed which pass continuously through both LV and OV. Neurons responding to tone bursts at long latencies (50-250 ms) are found dorsal, caudal and ventral to the tonotopic representations in LV and OV. Responses at such long latencies were rarely found in LV or OV. In general our data do not provide evidence for tonotopic organization of these long latency responses.

Within PO a low-to-high gradient of best frequencies is observed in a rostromedial-to-caudolateral direction. Neurons with similar best frequencies form bands oriented orthogonal to the frequency gradient. The caudolateral border of the high-frequency representation in PO adjoins the rostromedial border of the high-frequency representation of LV. The low-frequency representation occupies the rostral pole of PO. Thus, the tonotopic representation of PO appears to mirror to some extent the tonotopic representation of LV.

The pattern of thalamo-cortical connections is in register with tonotopic representation in the MGB and PO, as indicated by the correspondence between best frequencies of neurons in LV, OV and PO and the frequency representation at their targets in cortical fields A, AI and P.

94.20 THE TONOTOPIC ORGANIZATION OF THE AUDITORY GENICULOCORTICAL AND CORTICOPONTINE PROJECTIONS IN A CF-FM BAT. J. F. Olsen* (SPON: P.S.G. Stein). Dept. of Biology, Washington University, St. Louis, MO 63130.

The mustasched bat, Pteronotus parnelli rubiginosus, uses echolocation to orient and capture prey. Its auditory cortex is composed of several functional areas, some of which have been well defined physiologically. For example, Suga et al., have shown that neurons in the DSCF area respond optimally to a single frequency tone between 60 and 63 kHz, wheras FM-FM neurons show a facilitated response to paired frequency-modulated sounds (Science 203:270, 1979). Our previous work using tracer injections into these cortical areas has shown that the DSCF and FM-FM areas are connected with different, non-overlapping parts of the medial geniculate body (MGB). On this basis we proposed that there may be a fundamental difference in the origin of the geniculocortical projection to tonotopically versus non-tonotopically organized cortex (Fritz, Olsen, Suga and Jones. Soc. Neurosci. Abstr. 7:391, 1981). To further test this idea, small injections of WGA-HRP were made into the primary (tonotopically organized) auditory cortex under physiological guidance. The TMB method was used to reveal both anterograde and retrograde label. Injections of loci within the tonotopically organized 10 to 50 kHz area, located posterior to the DSCF area, resulted in labeled neurons within the most lateral part of the MGB. As the injection site is moved posteriorly within this cortical area, the retrogradely labeled neurons are found progressively more laterally and ventrally within the MGB. Geniculate neurons that project to the DSCF area are found in a medial part of the ventral division of the MGB, adjacent to, but segregated from the geniculate neurons that project to the remainder of primary auditory cortex. Those portions of the MGB that were found to project to tonotopically-organized cortex (including the DSCF area) are separate from those that project to non-tonotopically organized cortex (e.g., FM-FM area). This suggests to us that the "FM-FM" part of the MGB may be involved in the production of the complex stimulus sensitivity of cortical FM-FM neurons (i.e., facilitation). A neurophysiological study of the MGB is in progress to investigate the mechanism of this sensitivity.

We have also found a topographic order in the corticopontine projections from tonotopically organized cortex: Injections of the DSCF area produce patches of anterograde label located medially within the pontine nuclei, whereas injections of the 10 to 50 kHz cortical area labels portions of the lateral pons. In contrast, injections of the FM-FM area revealed a larger and more complex pontine projection which was distinct from that arising from tonotopically organized cortex. Supported by PHS research grant NS 17333 and BRSG (NIH SO7 RR 070 54-16).

95.1 OLFACTORY TUBERCLE'S DIFFERENT NEURAL ELEMENTS ACTIVATED BY LOCUS COERULEUS. L.P. Solano-Flores†, H.U. Aguilar-Baturoni and R. Guevara-Aguilar. Departamento de Fisiología, División de Investigación, Facultad de Medicina, U.N.A.M., Apdo.Postal 70250, México 20, D.F.

Olfactory tubercle (OT) evoked potentials were recorded following single pulse stimulation of the locus coeruleus (LC) in cats. Also, OT unit responses were recorded extracellularly following LC repetitive stimulation in rats. In order to characterize the properties of OT evoked potentials, these responses were evaluated following repetitive stimulation, paired shock, animal asphyxiation and damage to the recording site. Longer latency potential changes were more labile to repetitive stimulation,paired shock and asphyxiation than short latency changes. The time course of the effects following experimental procedures was not equal for the potentials of different latencies. Most of the individual OT neurons studied were not affected by repetitive LC stimulation. However, a small percentage were enhanced and the remainder decreased discharge frequency. Hypertensive effects were ruled out as a cause of the OT unit responses following LC stimulation because the temporal course of both events were not similar. Results of both electrophysiological techniques suggest that the LC influences different neural elements and/or neural populations within the OT. These responses appear to be independent of one another.

95.2 VISUAL ACUITY IN RATS: RAPID ASSESSMENT USING PATTERN REVERSAL EVOKED POTENTIALS. W. K. Boyes*†, R. S. Dyer, and W. E. Howell*. (SPON: L. W. Reiter). Neurotoxicology Division, Health Effects Research Laboratory, U. S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711.

The feasibility of using pattern reversal evoked potentials (PEPs) to assess visual acuity was investigated in albino and hooded rats. Assessment of visual acuity using behavioral methods establishes the limit of resolution at 0.34-0.44 cycles per degree (cpd) for albino rats, and at 1.2 cpd for hooded rats (Birch and Jacobs, 1978). Unfortunately, accurate behavioral tests often require months of training. PEPs have been used to rapidly assess spatial acuity in cats (Berkley and Watkins, 1971) and human infants (Sokol, 1977). PEPs have been recorded from rats at spatial frequencies up to 0.5 cpd (Onofrj et al., 1982), and have been used to determine refraction in rats (Meyer and Salinsky, 1977). In this study, PEPs to various sized patterns were used to create visual acuity functions in rats.

PEPs were recorded from previously implanted visual cortex electrodes referenced to the frontal area in awake restrained rats. Stimuli were phase reversing horizontal black and white bars.

PEP amplitude displayed an inverted U-shaped function with increasing spatial frequency. The increasing PEP amplitude from low to middle range spatial frequencies can be interpreted as a result of the increasing amount of edge in the display, and the decreasing amplitude at higher spatial frequencies as a function of the limits of spatial acuity. Sprague-Dawley albino rats showed increasing amplitude from 0.03 to 0.05 cpd, and decreasing amplitudes from 0.05 to 0.44 cpd. At 0.44 cpd, the PEP amplitude could not be discriminated from noise. The PEPs of Long-Evans hooded rats showed amplitude increases from 0.05 to 0.35 cpd and decreases from 0.35 to 0.64 cpd. Although the amplitudes remained above noise levels at 0.64 cpd, linear extrapolation suggested that the amplitude of the hooded rat PEPs would have declined to noise levels at 1.05 cpd. These data agree well with the behaviorally determined values.

Pilot data indicated that pupil dilation with atropine, which should decrease visual acuity by lowering the depth of the field of focus, decreased the amplitude PEP of hooded rats and shifted the peak of the inverted U-shaped function to the left to 0.18 cpd.

PEPs may therefore be useful for assessing visual acuity in rats.

†Supported by a National Research Council Research Associateship.

95.3 SPECTRAL ANALYSIS OF THE FREQUENCY FOLLOWING RESPONSE. R.L. Davis and R.H. Britt. Div. of Neurosurg., R155, Stanford Med Sch., Stanford, CA 94305 and Palo Alto Veterans Hospital, Palo Alto, CA 94304.

A series of lesion experiments were designed to define the neural generators that contribute to the frequency following response (FFR) in the cat. Spectral analysis was performed on farfield evoked responses differentially recorded between vertex-mastoid (bandpass filtered from 150 to 8K Hz). Histologically confirmed lesions were made in areas of the afferent auditory brainstem pathway by aspiration or radio-frequency thermocoagulation. Eleven msec duration tone burst stimuli were presented at a rate of 11/sec at frequencies ranging from 250 to 4K Hz. A one-third octave narrow band noise (NBN) centered at the stimulus frequency was mixed with the tone bursts in selected experiments in order to mask the neural response while preserving the cochlear microphonic.

Spectral analysis of the BAER and the FFR waveforms confirmed and extended amplitude analysis of the 500 Hz FFR described in Davis and Britt (Neuroscience Abstact, 7:282, 1981). Spectral activity was examined between 48.8 Hz and 6200 Hz with 48.8 Hz resolution. FFR spectra consisted of maximum energy at the stimulus frequency (fundamental) and reduced energy at the harmonics of the fundamental superimposed on a broad spectrum of low level activity (presumably from the response to the onset of the tone burst).The amount of activity present at the second and third harmonics relative to the fundamental showed promise as effective measure of the changes between the experimental ablation conditions independent of amplitude fluctuations. Results of this type of analysis suggest that each auditory area contributes components to the complexity of the response measured from the 500 Hz FFR. The largest contributions were detected from the ipsilateral superior olivary complex, the cochlear nucleus, the eighth nerve and the cochlear microphonic. Masking experiments supported ablation investigation findings that the complexity of the response originates from multiple generators. The frequencies which make up the unmasked 500 Hz FFR reduced to energy at 500 Hz only; no detectable response was observed at any other frequency when the tone burst was effectively masked with NBN.

The waveform complexity observed in the 500 Hz FFR is attributed to the contributions of multiple generators (ipsilateral superior olivary complex, cochlear nucleus, eighth nerve and cochlear microphonic). The simplified waveform resulting from ipsilateral masking with a narrow band noise suggested a single cochlear segment as the source of the response.

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95.4 Brainstem Auditory Evoked Potentials (BAEPs) in Rats as a Function of Gender, Stimulus Characteristics and Ethanol Sedation. M. W. Church*, H. L. Williams* & J. A. Holloway (SPON: G. A. McLean). Department of Psychiatry & Behavioral Sciences, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Human BAEPs vary as a function of developmental age, gender and stimulus characteristics. The present study investigated the influence of gender, stimulus intensity, rate and polarity on BAEPs in adult rats (15 males, 15 females).

Waveform. Rat BAEPs, recorded in response to 0.1 msec clicks through scalp electrodes, were comprised of 4 vertex-positive peaks occurring within 6 msec poststimulus. The principal neural generators of these waves are believed to be the auditory nerve (I), cochlear nucleus (II), superior olivary complex and trapezoid body (III) and the lateral lemniscus and/or inferior colliculus (IV).

Intensity. Rarefaction clicks (8/sec, 10-60 dB HL) were used to study the effects of intensity. Peak latencies decreased linearly as stimulus intensity increased with wave IV latency decreasing at a slower rate than waves I, II, and III. The wave I-IV interpeak latency (IPL) increased linearly with increasing stimulus intensity ($p < .001$). Wave amplitudes increased asymptotically with increasing stimulus intensity. Females, compared to males, had shorter latencies and IPLs, and larger amplitudes for waves I and II.

Rate. Rarefaction clicks (8, 60 & 120/sec, 60 dB HL) were used to study the effects of rate. All peak latencies increased linearly with increasing click rate ($p < .001$). Wave I changed the least, followed in order by waves II, III and IV. IPLs increased linearly with increasing click rate ($p < .001$). There were significant gender-by-rate interactions for wave IV and the I-IV IPL. Here, males had longer latencies than females at 8 clicks/sec but shorter latencies at 60 and 120 clicks/sec. Wave amplitudes decreased asymptotically with increasing click rate.

Polarity. Sixty dB HL clicks (8/sec) were used to study the effects of polarity. All peak latencies were shorter in response to rarefaction (R) than condensation (C) clicks (significant for waves I and II). The I-IV IPL was shorter in response to C clicks but not significantly. Wave II amplitude was smaller in response to C clicks ($p = .004$).

Ethanol. Rectal temperatures decreased 0.2 ± 0.2^{o}C (males) and 0.2 ± 0.5^{o}C (females) while the I-IV IPL increased 0.09 ± 0.01 and 0.06 ± 0.10 msec following ethanol injection (1.5-2.0 g/kg, i.p.). There was no relation between temperature and I-IV IPL changes except in 2 animals with temperature decreases exceeding 0.4^{o}C.

95.5 DEVELOPMENT OF BINAURAL INTERACTIONS IN AUDITORY BRAINSTEM EVOKED RESPONSES. C. Shipley,* J. Strecker* and J. Buchwald. Dept. of Physiology, Brain Research Institute, Mental Retardation Research Center, University of Calif. Medical Center, Los Angeles, CA 90024.

Binaural interactions in auditory brainstem evoked responses (ABRs) are reflected in differences between the ABR obtained from binaural stimulation and the ABR constructed by adding the results of separate monaural stimulations to each ear. Typically, the summed monaural response is larger than the binaural response for some waves of the ABR indicating that differences exist between monaural and binaural signal processing in the neural generators of these waves.

In the present study, monaural and binaural ABR responses were recorded and compared for six adult cats and six kittens to determine the form and developmental sequence of binaural interactions in the cat ABR. Stimuli were .1 msec clicks delivered through miniature speakers in a set of cat headphones specifically designed to reduce acoustic cross-over between the ears. The level at which acoustic cross-over evoked an ABR in this situation was studied in pilot tests by surgically deafening monaurally, stimulating the deafened ear, and observing what click intensity produced an ABR by cross-over to the normal contralateral ear.

Binaural interactions which could not be attributed to acoustic cross-over were reliably observed in adults at the latencies of the fourth, fifth and sixth ABR waves. In addition, a small but reliable positivity was seen at a latency intermediate between ABR waves 4 and 5 that was consistently larger for monaural than for binaural stimulation.

A statistic reflecting the percent of binaural interaction was calculated by dividing the amplitude of the difference potential (summed monaural minus binaural potentials) associated with each peak in the ABR by the amplitude of that peak in the binaural stimulation condition. The average value of this statistic increased with successive waves from 4 (55%) to 5 and 6 (80-90%), suggesting that later waves of the ABR may be increasingly involved in the processing of binaural information.

The ABR binaural interactions were present in kittens as young as 20 days of age. It was difficult to test the response before this age because of differences in the thresholds of the two ears, probably due to slightly different rates of ear opening. The percent of binaural interaction was at adult levels for wave 4 in kittens 20-30 days of age. The percent of binaural interaction at wave 5 tended to be smaller than that seen in adults; this difference approached but did not reach statistical significance. Wave six was not present in animals of this age.

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95.6 AN ANIMAL MODEL FOR STUDYING NEAR FIELD, FAR FIELD AND SPINAL CORD SOMATOSENSORY EVOKED POTENTIALS. J.G. Blackburn*, S. Trojanowski*, C.D. Lusk*, and S. Katz. Dept. of Physiology, Medical University of S.C., Charleston, S.C. 29425.

Near field somatosensory evoked potentials (SEPs) have been used extensively in the evaluation of spinal trauma. In recent years, investigators have also begun to use far field (FFPs) and spinal cord (SCPs) evoked potentials for diagnostic purposes.

The present study describes a model for recording all three potentials (SEPs, FFPs and SCPs) simultaneously in the same animal. Adult cats were anesthetized with ketamine HCl (10 mg/kg, IM). Following intubation, anesthesia was maintained with 70% N_2O and 30% O_2. The animals were paralyzed with pancuronium bromide (0.1 mg/kg, IV) in order to eliminate movement artifacts from the recordings. The left superficial peroneal nerve was stimulated percutaneously with constant current square waves (8-10 mA, 0.3 msec duration). Evoked potentials were recorded from an epidural electrode over the contralateral leg area of the somatosensory cortex (SEPs and FFPs) and percutaneous electrodes over the cervical and thoracic areas of the spinal cord (SCPs). Recordings were monopolar with the ears being used as reference (both ears were electrically interconnected). Bipolar recordings were also taken from the spinal cord. The potentials were amplified, filtered (1-3 KHz for SEPs and SCPs, 0.3 to 3 KHz for FFPs), summated with a microprocessor (varying number of sweeps) and displayed on a digital plotter. Electrocardiographic (EKG) contamination of all potential recordings was eliminated by triggering the stimulator and microprocessor from the EKG. Stable, well-defined SEPs, FFPs, and SCPs were recorded over an extended period of time. The ability to record these potentials simultaneously is important in studies of spinal trauma when the electrophysiological condition of the cord is continuously changing following impact trauma. The EKG, EEG and end-tidal % CO_2 were recorded as indices of the physiological state of the animal. Any animal displaying significant changes in these physiological parameters during the course of the experiment was eliminated from the study.

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95.7 SHORT LATENCY TRIGEMINAL SOMATOSENSORY EVOKED POTENTIALS IN THE RAT: POSSIBLE ANATOMICAL GENERATORS. K. L. McKeown, B. Budnick* and W. C. Wiederholt. VA Hospital, San Diego, CA 92161; Department of Neurosciences (V151), University of California, San Diego, La Jolla, CA 92093.

Averaged somatosensory evoked potentials (SEPs) following stimulation of the maxillary branch of the trigeminal nerve were recorded from acute, anesthetized rats. We consistently recorded a series of five potentials preceding the primary cortical component of the SEP. We correlated surface and depth activities by recording SEPs simultaneously from skull electrodes and from depth electrodes placed in the trigeminal tract, VPM nucleus of the thalamus, thalamic radiation and primary cortical receiving area for the face. Latencies and amplitudes of surface and depth recorded potentials, responses to increasing rates of stimulation and effects of lesions were analyzed. These observations suggest that activity in the trigeminal nerve contributes to the first surface component, that activities in the trigeminal nerve and brainstem nuclei contribute to the second surface component, that activities in the trigeminal tracts contribute to the third surface component, that activity in the VPM nucleus contributes to the fourth surface component, and that activity in the thalamic radiation contributes to the fifth surface component.

95.8 SOMATOSENSORY, VISUAL, AND AUDITORY EVOKED POTENTIALS IN MONKEYS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE). J. C. Slimp and E. C. Alvord, Jr.*. Depts. of Rehabilitation Medicine and Pathology, Univ. of Washington Sch. of Med., Seattle, WA 98195.

Evoked potentials (EPs) are being used more and more frequently in the clinical diagnosis of central nervous system disease. CNS demyelinating diseases such as multiple sclerosis and its experimental analog, EAE, are particularly amenable to diagnosis by EPs.

Our previous work on a small number of EAE monkeys tested while awake (Slimp, J.C., Muscle and Nerve, 4:439, 1981) and a larger sample of EAE monkeys tested under anesthesia (presented at American Association of Electromyographers and Electrodiagnosticians meeting, 1982) established a strong correlation between EP abnormalities and the occurrence of EAE. The temporal course of abnormal EPs with EAE in awake tested monkeys suggested an onset of abnormal EPs coincident or prior to clinical EAE and perhaps in the absence of clinical symptoms.

Our recent study addressed the question of early diagnosis of EAE by EPs. Sixteen (16) Macaca fascicularis monkeys were challenged with porcine myelin basic protein and Freund's adjuvant to induce EAE. Recordings of somatosensory, visual, and auditory EPs were made with subcutaneous needle electrodes under Vetalar/Rompun anesthesia. Two series of four (4) consecutive days of recordings were done, one series prior to the challenge and the second series at two (2) weeks following the challenge. If EAE had not occurred during the tests at two (2) weeks post challenge, recordings were taken at least every other day until four (4) weeks post challenge.

Ten (10) monkeys developed clinical EAE characterized by typical symptoms of paresis or paralysis, tremor, head rotation, nystagmus, and dilated pupils. All ten (10) of these animals had abnormal EPs in one or more modalities. Changes in somatosensory and auditory EPs were more often observed than changes in visual EPs. Most interestingly, abnormal EPs were observed on or before the onset of clinical EAE in 80% of these animals.

Furthermore, of significance was the observation that in four (4) of the six (6) monkeys that showed no clinical symptoms, abnormal EPs were present on several occasions during the third week post challenge.

These results confirm our preliminary observations that EPs can indicate early onset and subclinical attacks of EAE. The correlation of EP data with analyses of blood and spinal fluid is under investigation. These data have particular bearing upon the clinical sue of EPs in the diagnosis of multiple sclerosis.

95.9 ELECTROPHYSIOLOGIC EFFECTS OF MORPHINE AND NALOXONE, J. B. Myklebust and J. F. Cusick (SPON: H. Myklebust). Dept. of Neurosurg., Medical College of Wisconsin, Milwaukee, WI 53226 and V.A. Medical Center, Wood, WI 53193

Since epidural administration of morphine is used for chronic pain, studies have been conducted using an animal model devised for the evaluation of epidural anesthetics.[1] Since naloxone was used as a morphine antagonist, and because of recent reports of therapeutic benefit,[2] studies have also been conducted on the effects of naloxone alone and in conjunction with morphine.

Studies were conducted in 5 stumptail monkeys (8-10 kg). Electrodes were placed dorsally over the conus medullaris and upper thoracic spinal cord, bilaterally over the sensorimotor cortices, and in thalamic nuclei centre median and ventralis posterolateralis. Evoked potentials were recorded secondary to peroneal nerve stimulation. 0.2 mg morphine was injected into the epidural space at T12. Following the morphine injection, 0.4 mg naloxone was injected intravenously. In separate studies, up to 2 mg of naloxone was injected intravenously.

Epidural morphine eliminated the large monophasic portion of the dorsal root entry zone response (probably reflecting segmental gray matter activity), leaving the early inflections intact (reflecting activity in the dorsal roots and primary afferent fibers). The response from the thalamic intralaminar nuclei was abolished; however, the response from rostral spinal levels, nucleus VPL, and sensorimotor cortex was intact. Intravenous injection of naloxone reversed the effects within 15-30 minutes.

Intravenous injections of up to 2 mg of naloxone produced transient 50-100% increases in the amplitude of the cortical evoked potential. The effects of subcortical and spinal levels were less pronounced. The effects lasted 10-15 minutes, appeared to be dose related, and were reproducible with repeated injections. These effects were not reversed by intravenous morphine injections. In parallel studies in the cat, similar effects were noted in the cortical response. Additionally, a 10-20% increase in local cerebral blood flow was measured using a combination isothermal hydrogen clearance flow probe.[3]

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96.1 AMPHETAMINE LOWERS BODY WEIGHT SET POINT: IMPLICATIONS FOR UNDERSTANDING TOLERANCE TO AMPHETAMINE ANOREXIA. David L. Wolgin. Dept. Psychol., Florida Atlantic University, Boca Raton, Fla. 33431.

Rats maintained on a deprivation schedule of feeding were given daily injections of d-amphetamine sulfate (2 mg/kg) and either milk, milk adulterated with quinine (.06 mg/cc), or Purina Lab Chow pellets, for 30 min. Control rats were given daily injections of saline, but otherwise treated similarly. Amphetamine-injected rats initially lost weight, and then maintained their weight below the levels of saline controls. The degree of weight loss varied with the diet: Rats given milk maintained their weight at a higher level than those given either of the other two diets. No tolerance was observed to the weight suppressant effect of the drug.

In contrast, analysis of food intake revealed a pattern of initial anorexia and subsequent recovery, typically thought to reflect tolerance to the drug. The rate of recovery of intake varied with the diet: Rats given milk recovered more quickly than rats given either adulterated milk or pellets. When such apparently tolerant rats were switched from milk to either of the other two diets, intake was chronically suppressed and body weight dropped to a lower level. When switched from adulterated milk or pellets to milk, intake was chronically elevated and body weight rose to a higher level. The differential changes in intake brought about by changing the diet are difficult to reconcile with the view that the rats were tolerant to the drug.

Instead, the data suggest that amphetamine lowers the set point for body weight regulation, an effect that does not show tolerance (cf. Stunkard, In: Anorectic Agents: Mechanisms of Action and Tolerance, ed. by S. Garattini and R. Samanin, Raven Press, New York, 1981, pp. 191-209). From this perspective, the initial anorexia produced by the drug can be viewed as a mechanism to achieve a lower weight level, while recovery of intake represents the maintenance of the new weight level, not tolerance to the drug. Because the set point for body weight is modulated by palatability, as in undrugged rats (see Corbit & Stellar, JCPP, 1964, 58, 63-67; Powley & Keesey, JCPP, 1970, 70, 25-36; Keesey & Boyle, 1973, 84, 38-46), changing to a more palatable diet evokes increases in food intake to achieve a higher weight level, while changing to a less palatable diet evokes decreases in intake to achieve a lower weight level.

96.2 CHOLINERGIC EFFECTS IN TARDIVE DYSKINESIA: ARE THERE SUBTYPES? H.A. Nasrallah, R.E. Smith*, F.J. Dunner*, M.K. McCalley-Whitters* and A. Sherman*. VA Medical Center and Dept. of Psychiatry, University of Iowa College of Medicine, Iowa City, IA.

Tardive dyskinesia (TD) is a frequently irreversible disorder of oro-lingual, peripheral or trunkal choreiform involuntary movements resulting from chronic neuroleptic treatment. The pathophysiological mechanism of TD has been proposed to be a state of relative hyperdopaminergic/hypocholinergic imbalance in the nigro-striatal tract secondary to chronic chemical denervation by neuroleptic drugs, which are dopamine-receptor blocking agents. Thus, dopaminergic antagonists or cholinergic agonists would be expected to improve the symptoms of TD. Choline is a physiological precursor of acetylcholine, and a few reports in the literature on a total of 34 subjects indicated that choline administration produced improvement in TD. Here, we present a double-blind crossover study of choline chloride in TD.

Ten chronic psychiatric patients (6 males, 4 females) with TD of 2-4 years duration consented to participate in the study on a research ward. Placebo capsules of choline chloride were given q.i.d. for 2-3 weeks, followed by 4 weeks of active drug (200-300 mg/kg/day of choline chloride), and finally 3 weeks of placebo again. Each patient was videotaped weekly throughout the study, and the video-segments edited and randomized. Two psychiatrists blind to the design rated the weekly changes in TD symptoms using the Abnormal Involuntary Movement Scale (AIMS). Blood was drawn at the end of every drug phase to determine serum choline and acetylcholine concentrations.

Seven patients showed clinical improvement in TD during the active drug phase. Two other patients showed minimal improvement and one patient did not change. However, a Wilcoxon Rank Sum Test of the AIMS scores before, during and after choline administration was not significant. There was however a significant increase in both choline and acetylcholine serum concentrations in the entire sample during the active drug phase.

The results suggest that despite the noticeable clinical improvement observed in many patients with choline chloride, the overall change was not statistically significant. This may be due to the fact that some patients showed only mild or no improvement on blind ratings. This suggests the possibility of subtypes of TD, with one subtype conforming to the expectations of a hyperdopaminergic/hypocholinergic model while another subtype may not. The lack of consistent results in the literature with other cholinergic agonists (physostigmine, lecithin) support the notion of TD subtypes.

96.3 Valproic acid induced alteration of zinc and selenium status. R.W. Hurd,* B.J. Wilder, H.A. Van Rinsvelt,* B.J. Karas,* E.A. Perry* and W. Maenhut*. Depts. of Neuroscience and Physics, University of Florida, Neurology Section, V.A. Medical Research Center, Gainesville, FL, and Inst. for Nuclear Sciences, Univ. of Ghent, Belgium.

Several lines of evidence implicate a role for zinc in the epileptogenic process: (1) Zinc is a potent inhibitor of Na+-K+-ATPase and i.c.v. injections of small quantities produce convulsions; (2) High zinc levels inhibit GAD, GABA-T and SSA-DH activity suggesting a regulatory role in GABA synthesis; (3) Seizure activity in the photically sensitive Papio papio baboon is diminished by chronic zinc chelation therapy; (4) Zinc levels are decreased by phenytoin.

A number of side effects seen in patients on valproic acid (VPA) therapy are often seen with zinc and other metal deficiencies. These include gastrointestinal disturbance, drowsiness, tremor, ataxia, anorexia, hyperammonemia, hepatotoxicity and pancreatitis. In addition, high dosage VPA and zinc deficiency produce testicular and thymic atrophy in experimental animals.

We have previously shown that VPA binds zinc by Differential Pulse Polarogrophy (Neurosci. Abst. 7: 813, 1981). In present studies, VPA was administered 150 mg/kg i.p. twice a day for 7 days to 10 rats and plasma and liver metals analyzed. Ten control animals received i.p. saline. Analysis of metals was performed by Particle-induced X-ray Emission (PIXE) using the Van de Graaf accelerator at the University of Florida and the compact isochronous cyclotron at the Institute for Nuclear Science, at the University of Ghent. Animals receiving VPA had significant depletion of plasma zinc ($p < .05$) and selenium ($p < .02$, student's t-test). There was a 30% reduction in hepatic selenium ($p < .05$). Thymic weight was decreased (.418 ± .035 g for controls, .222 ± .013 g for VPA group). Plasma and liver K, Ca, Fe, Mn, Cu, Br or Rb were not altered.

In a related study plasma metal content from 6 patients receiving VPA as sole anticonvulsant therapy and 9 control subjects was determined by PIXE. In comparison to controls, plasma selenium levels were significantly decreased in patients taking VPA ($p < .05$). Two patients exhibiting drug associated tremor activity had low levels of copper (58 and 63 µg/dl, control: 101 ± 12 µg/dl).

We conclude that changes in body metal status, particularly selenium, zinc and copper, could well contribute to undesired side effects of VPA therapy.

96.4 EFFECT OF ACUTE AND CHRONIC ACRYLONITRILE EXPOSURE ON METRAZOL-INDUCED SEIZURES IN THE RAT. D. Fanini*, N. Trieff*, A. Ahmed* and P. Adams. Department of Preventive Medicine, Department of Pathology, and Department of Psychiatry & Behavioral Sciences, Univ. of Texas Medical Branch, Galveston, Texas 77550.

The effects of acute and chronic exposure to acrylonitrile (10-40 mg/kg, gavage) on the latency and duration of metrazol-induced seizures were studied in adult, male Sprague-Dawley rats. Low dosages of acrylonitrile (10 and 20 mg/kg) which alone produce no apparent signs of toxicity were effective in increasing the latency to onset of metrazol-induced seizures and resulted in shorter seizures. Higher nonlethal dosages of acrylonitrile resulted in long latency but severe seizures generally resulting in death. The results suggest a biphasic interaction of acrylonitrile with metrazol. At lower dosages acrylonitrile protects against seizure induction while at higher dosages there is potentiation producing a lethal effect. Possible mechanisms discussed include involvement of a cholinomimetic action of acrylonitrile, the role of cyanide (a by-product of acrylonitrile metabolism), and hypoxia. Supported in part by grant ES 01871.

96.5 BRAIN MONOAMINE TURNOVER FOLLOWING D-AMPHETAMINE IN LEAD-EXPOSED RATS. S.M. Lasley, R.D. Greenland*, D.J. Minnema*, and P.M. McGinnis*. Dept. Env. Hlth., Univ. Cinti. Coll. Med., Cinti, OH 45267.

The results of attempts to identify CNS neurochemical changes following low level lead (Pb) exposure in developing animals have been equivocal. In contrast, a frequently reported observation is that Pb-exposed rodents exhibit altered behavioral responses following d-amphetamine (AMPH). Thus, several endpoints of monoamine metabolism were measured in drugged and non-drugged Pb-exposed and control animals to evaluate basal neurotransmitter function as well as the transmitter response to the CNS stimulant.

At parturition Long-Evans dams received 0.2% Pb acetate in the drinking water thus exposing the suckling pups to Pb via maternal milk. Control dams received distilled water. Pups were weaned to, and maintained on, the same solution given their dam until sacrifice. At 110-120 days a chronic indwelling jugular catheter was implanted in each animal for the precise administration of 1.0 mCi L-[ring-2,6-^{3}H]-tyrosine and 0.5 mCi L-[^{3}H(G)]-tryptophan. Forty min after precursor administration animals received either 1 mg/kg s.c. AMPH or saline and were sacrificed 20, 50, 80, or 140 min later. Brain regions were dissected and the biogenic amines, precursors, and metabolites extracted and quantified by HPLC with electrochemical detection. Radioactivity in chromatographic fractions was quantified by liquid scintillation spectrometry.

Day 90 blood Pb (43.1 µg/dl) and free erythrocyte protoporphyrin (91.7 µg/dl) values were consistent with a low body burden of the metal in Pb-exposed animals. Nevertheless, Pb animals displayed an altered neurochemical response to AMPH as reflected by changes in the endogenous concentrations and specific activities of dopamine (DA). DA and 3-methoxytyramine (3-MT) levels in striatum and nucleus accumbens of Pb rats given AMPH were significantly elevated above drugged controls at 20 min post-drug, were similar to controls at 50 min, and remained significantly increased above baseline values until 80 min post-injection. In Pb animals receiving AMPH DA specific activity declined at a greater rate in these two regions than in drugged control rats, an additional indication of increased DA utilization after AMPH as a result of chronic Pb exposure. In contrast, non-drugged animals exposed to Pb displayed decreased DA and 5-HT turnover rates and concentrations of 5-hydroxyindoleacetic acid. These findings describe a Pb-induced deficit in neuronal function that is also exhibited in an altered response to a pharmacologic stimulus. The interrelationships of DA and 5-HT may be important in understanding the AMPH-induced behavioral changes seen in Pb-exposed animals (Supported by NIH grants ES-01566 and 05146 and EPA grant 805693.)

96.6 REGULATORY AND LOCOMOTOR CONSEQUENCES OF TRIMETHYLTIN POISONING IN RATS. C.T. Johnson*, A.R. Dunn*, C.J. Robinson*, T.J. Walsh, and H.S. Swartzwelder. Institute of Animal Behavior, Towson State Univ., Towson, Md.

Trimethyltin chloride(TMT Cl) administration has been shown to produce both neuropathological and behavioral changes. The facts that (a) the neuronal damage involves preferentially the limbic system and (b) the behavioral changes are reminiscent of those observed in animals with non-chemically induced hippocampal lesions led the present investigators to extend the comparison by viewing the effects of TMT Cl poisoning on a number of behaviors over the course of approximately four months. The subjects were 25 male hooded rats, each of which received one of three substances by intragastric intubation at 95 days of age: 7 mg/kg TMT Cl, 5 mg/kg TMT Cl, or .9% NaCl. TMT Cl dosage was calculated as the base and administered in a volume of 1 ml/kg body weight. Open field behaviors and spontaneous alternation were tested during four separate five-day periods throughout the experiment: prior to injection, and days 14-18, 56-60, and 106-110 postinjection. During these five-day periods, spontaneous alternation was tested once a day; open field behaviors were tested twice during each period, once during the light cycle and once during the dark cycle. Throughout the entire experiment, body weights were taken every third day and water intakes were measured daily. In support of previous studies, open field activity was found to be significantly greater in animals treated with 7 mg/kg TMT Cl than in animals treated with NaCl. Animals treated with 5 mg/kg TMT Cl were intermediate in activity. However, there were no group differences in rearing, grooming, or defecation, nor were there any differential results from light versus dark cycle testing. Average postinjection spontaneous alternation rates were higher in the control animals than in the experimental animals, but the difference was not statistically significant. Both groups of experimental animals showed significantly greater water intake than the control animals, especially during the first 21 days postinjection. Finally, the animals treated with 7 mg/kg TMT Cl exhibited a significant interruption of weight gain, although this interruption persisted generally for less than one week. These data will be discussed in terms of their relationship to the literature on the effects of non-chemically induced hippocampal damage.

96.7 EFFECTS OF TRIETHYLTIN (TET) ON MODULATION OF THE ACOUSTIC STARTLE RESPONSE IN RATS. L.D. Fechter, G. G. Bierkamper, & J. S. Young. Dept. of Environmental Hlth. Sciences, Johns Hopkins University Baltimore, MD 21205

Triethyltin (TET) intoxication produces a variety of neuromuscular consequences including slowing of nerve conduction velocity (NCV), hindlimb weakness and deficiencies in evoked release of ACh. Histological studies suggest that changes in NCV may be due, in part, to myelin-splitting in proximal and spinal regions of peripheral nerves. While it has previously been shown that TET exposure significantly depresses startle reactivity, it is not clear whether this effect reflects a motor deficit or whether it might result from a toxic effect of TET on the auditory system. The present experiment evaluates the startle response in rats whose neuromuscular system is impaired by TET exposure. We were specifically interested in the contribution of the peripheral musculature to the auditory startle reflex and our ability to detect a sensory impairment in the presence of such a motor dysfunction.

Adult male Long Evans rats were pretested to determine auditory thresholds by the method of startle reflex modulation (Young & Fechter, in press). Briefly, low intensity pure tones are presented prior to elicitation of a startle reflex by a sudden white noise burst under conditions designed to inhibit the reflex. Since reflex inhibition is directly related to intensity of the pure tone prestimulus, it is possible to generate a function relating increasing reflex inhibition to increased intensity of the prestimulus. A threshold for hearing is defined as that intensity prestimulus which produces just significant reflex inhibition relative to a baseline response obtained on trials not containing prestimuli. Since determination of the sensory threshold is based upon a baseline response, detection of threshold shifts should be independent of shifts in the baseline response. However, this has not been tested. Experimental subjects were given TET (30 mg/ml) in their drinking water and were retested daily starting two days later. Control subjects were placed on a reduced water regimen which matched the water intake of the experimental animals.

A dramatic reduction in acoustic startle reflex behavior was apparent in all experimental subjects on the first test following TET exposure. Despite this reduction, audiometric testing conducted with test stimuli of 10 and 40 KHz indicated no shift in auditory thresholds.

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96.8 EFFECTS OF NEUROTOXIC DIAMINES ON RAT BRAIN ACETYLCHOLINESTERASE. W. Flory, M. L. Marceau-Day* and G. M. Strain. Vet. Physiol., Pharmacol., Toxicol., Louisiana State Univ., Baton Rouge, LA 70803.

Simple aliphatic diamines, when administered to the brain ventricles in micromolar quanitities, produce depression, seizures, or both, followed by death (Strain and Flory, Soc. Neurosci. Abstr. 6:149, 1980). The compounds have been shown to weakly inhibit rat brain and liver monoamine oxidase (Flory and Strain, Fed. Proc. 41:646, 1982), but the pattern of inhibition did not parallel the observed in vivo toxicity. We have now studied the effects of the diamines on acetylcholinesterase (AChE).

AChE activity was quantified using a crude rat brain homogenate, with acetylthiocholine iodide as substrate. Hydrolysis of the substrate was detected by a spectrophotometer at 405 nm, with a reaction coupled to dithiobisnitrobenzoic acid. Kinetic curves were determined for controls and in the presence of the diamines ethylenediamine, 1,2-diaminopropane, 1,3-diaminopropane, 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane, and 1,7-diaminoheptane. Curves were obtained in duplicate from nine levels of substrate and at four diamine levels.

For this system, calculated control parameters were K_m = 32.7 µ molar and V_{max} = 0.991 measured as Δ O.D./mg/min. Diamine effects on AChE were similar for all compounds tested. Increased enzyme inhibition was observed with lengthening carbon chains; 1,7-diaminoheptane and 1,6-diaminohexane were the most inhibitory. All diamines showed a mixed pattern of inhibition. At low substrate concentrations, there was a decrease in activity, while at high substrate levels there was an apparent enzyme activation. The in vitro enzyme inhibition pattern did not parallel the observed in vivo neurotoxicity. (Supported by LSU-SVM Organized Research Grants 179 and 247.)

96.9 LONG-TERM EFFECTS OF NEONATAL LEAD EXPOSURE ON HAMILTON SEARCH TASK AND DELAYED SPATIAL ALTERNATION IN THE MONKEY. E. D. Levin* and R. E. Bowman. Psychology Primate Lab., University of Wisconsin-Madison, Madison, WI 53706.

Six-year old Rhesus monkeys were tested on the Hamilton search task (HST) and delayed spatial alternation (DSA). Three monkeys had been exposed to an average of 0.5 mg./kg./day of lead acetate in their drinking water for the first year after birth. Two monkeys were untreated controls. We previously reported (Levin & Bowman, Neurosci. Abstr. 6: 170, 1980) that postnatal exposure of the monkey to lead later resulted in a significant deficit in HST. The lead-treated monkeys in the present experiment received, on a different schedule, lead levels between those given to the groups in the previous experiment, but they did not show a significant deficit in HST performance. In the HST the monkeys must search through 8 boxes to find food rewards. In the DSA test, the monkeys had to alternate their responses between right and left hand stimuli on successive trials to receive food rewards. Delays of 5, 10, 20 and 40 seconds were interposed between trials. An initial preference trial and 32 delay trials (8 of each delay) were run in each session. The percent correct trials were calculated for each delay. The sessions were blocked in groups of four to improve the stability of the measure.

There was a significant main effect of lead ($p < .025$) with the lead group performing worse than the controls. Averaged over all delays and blocks the controls had 73.0 % correct and the lead group had 56.0 % correct. The control group's % correct scores were consistantly higher than the lead group's in each block. The main effect of blocks was significant ($p < .05$), but there was no significant lead X blocks interaction. The lead-induced deficit was most apparent at the 5-second delay but was present at all delays. Since the deficit did not seem to worsen with longer delays, we concluded that the lead-induced deficit was not with memory per se but probably with attention or encoding processes. These data fit well with the rat literature on lead toxicity reporting behavioral (Alfano & Petit, Behav. & Neural Biol. 32: 319-333, 1981) and morphological (Alfano et al., Exp. Neurol. 75: 308-319, 1982) indications of lead-induced hippocampal impairment. Hippocampal lesions have been shown to impair DSA performance in the monkey (Waxler & Rosvold, Neuropsychologia 8: 137-146, 1970). This study indicates that DSA is a very sensitive task in detecting the long-term toxic effect of neonatal lead exposure on cognitive functioning. Given that the lead exposure stopped 5 years before the start of testing, this deficit in cognition seems to be permanent. (Supported by NIH grant ES01062 and the Food Research Institute, Madison, WI)

96.10 LACK OF CROSS-TOLERANCE BETWEEN SYSTEMIC AND INTRATHECAL MORPHINE IN RATS ON THE NOCICEPTIVE TAIL FLICK TEST. C. Advokat and C. Tyler*. University of Illinois College of Medicine, Chicago, IL. 60612

The analgesic effect of intrathecal (spinally administered) narcotics has been demonstrated in both, laboratory animals and in acute and chronic pain patients. There is, however, a major difference between the experimental and clinical results. The scant evidence available suggests that laboratory animals who become tolerant to systemic opiates are cross-tolerant to intrathecal opiates. In contrast, chronic pain patients, with a history of narcotic medication, are usually analgesic in response to intrathecal opiates at the same doses which are effective in nontolerant, acute (postoperative) pain patients. Moreover, a number of reports have shown minimal if any, tolerance in humans after several weeks or months of chronic spinal opiate administration. To examine more closely this apparent absence of cross-tolerance the present experiments assessed the antinociceptive effect of intrathecal morphine in rats made tolerant to systemic morphine.

Male rats weighing approximatley 400 g were implanted with intrathecal catheters by the method of Yaksh and Rudy (Phys. Behav. 17: 1031, 1976). After 2 weeks of postoperative recovery, tolerance was induced either by daily systemic injection of morphine sulfate (3 mg/kg) or by implantation of morphine pellets (3 pellets, each containing 75 mg of morphine base). Behavioral tolerance to opiate analgesia, assessed by the tail flick test (TF) to noxious thermal stimulation, developed during the course of chronic morphine administration. When TF latencies of morphine and placebo treated rats no longer differed, all animals were injected intrathecally with various doses of morphine. Regardless of whether tolerance was induced by chronic injection or pellets, morphine and placebo rats were equally analgesic to intrathecal opiate administration. Intrathecal injection of the vehicle, saline solution, did not produce analgesia. In addition, naloxone (0.4 mg/kg) elicited significantly more "wet-dog shake" withdrawal responses in morphine as opposed to placebo implanted rats. These data suggest that tolerance to (and dependence on) systemic opiates need not reduce the efficacy of spinal opiates. The data are consistent with clinical reports which indicate that intrathecal morphine is analgesically effective in patients who are tolerant to systemic narcotics. (Supported by intramural, Biomedical Research Support Grant PHS BRSG 81X03).

96.11 STIMULUS CONTROL OF TOLERANCE TO d-AMPHETAMINE. M.W. Emmett-Oglesby, D. Wood*, H. Lal and D.G. Spencer. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

This experiment tested whether tolerance to d-amphetamine (d-A) is confined to a task in which it is trained. Rats were trained to perform two tasks which alternated on a daily basis. One task was lever-pressing under a differential-reinforcement-of-low-rate 10-sec (DRL 10) schedule of food reinforcement; the other task was consumption of sweetened-milk. Dose-effect data were determined for both tasks in all rats: d-A decreased the quantity of milk consumed and the number of reinforcements earned on the DRL 10 in a dose-dependent manner. These data were used to assign rats to three groups which did not differ significantly from each other in their initial dose-response curves in either task: group SAL received saline prior to both tasks; group MILK received d-A, 2.5 mg/kg, prior to milk consumption and saline prior to the DRL 10, and group DRL received d-A, 0.625 mg/kg, prior to the DRL 10 and saline prior to milk consumption. These conditions were maintained for 64 sessions (32 drug exposures for the DRL and MILK groups), by which time tolerance had developed to the disruptive effect of d-A in both tasks, and then dose-effect data were redetermined in all rats. The DRL group was significantly less disrupted in the DRL 10 than either the MILK or SAL groups, which did not differ from each other. The MILK group was significantly less disrupted in the milk-drinking task than either the DRL or SAL groups, which did not differ from each other. Thus, tolerance is under stimulus control because animals tolerant to d-A in one paradigm are not tolerant in a task where they have no history of chronic exposure to the drug. (Supported by Faculty Research Grant #34940).

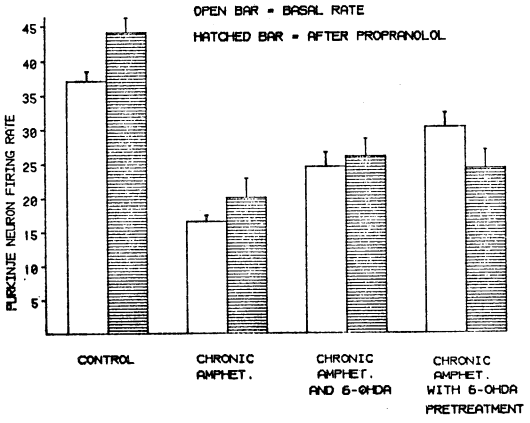
96.12 EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF HALOPERIDOL ON BIOCHEMICAL CHARACTERISTICS OF DIFFERENT DOPAMINERGIC NEURONS IN MALE AND FEMALE RATS. K.E. Moore, G.D. Riegle* and K.T. Demarest. Depts. of Pharmacol./Toxicol. and Physiol., Michigan State Univ., East Lansing, MI 48824.

Acute administration of neuroleptics to rats causes a prompt increase in serum concentrations of prolactin and an increase in the activity of major ascending dopamine (DA) neurons, as estimated from rates of synthesis and turnover of DA in terminal regions of these neurons [nigrostriatal terminals in striatum (ST); mesolimbic terminals in nucleus accumbens (NA) and olfactory tubercle (OT)]. Neuroleptics also cause a delayed (12-16 h) activation of tuberoinfundibular DA neurons, as estimated from rates of synthesis and turnover of DA in the median eminence (ME); this effect is mediated by the neuroleptic-induced increase in serum concentrations of prolactin.

The object of the present experiments was to monitor these same effects in male and female rats during chronic administration of a potent neuroleptic, haloperidol. Male Long-Evans rats were injected daily with haloperidol (1 mg/kg, sc) or its vehicle (0.3% tartaric acid) for up to 11 days. The animals were sacrificed at various times during treatment and 24 h after the last injection (day 12). The concentration of DA in forebrain regions did not change during the experiment, but the rate of DA synthesis (DOPA accumulation after the administration of 3-hydroxybenzylhydrazine, 100 mg/kg, ip, an inhibitor of aromatic L-amino acid decarboxylase) significantly increased within 2 h after the first injection, remained elevated during the course of the injections, and returned to or below control values on day 12. Tolerance developed to the effect of haloperidol on DA synthesis in ST and to a lesser extent in OT and NA. The concentration of DA in the ME was significantly reduced after 3 injections of haloperidol and remained low thereafter. The rate of DA synthesis in ME was not increased 2 h after the first injection of haloperidol, but by 24 h it was increased and thereafter it remained high even up to 24 h after the last injection. The serum prolactin concentration increased and the DA content of the adenohypophysis decreased within 2 h after the first injection; no tolerance developed to these effects, and complete recovery occurred on day 12. Thus, tolerance develops to the effects of haloperidol on DA synthesis in terminals of nigrostriatal but not tuberoinfundibular neurons, and no tolerance develops to the effects of haloperidol on the adenohypophysis (DA content and prolactin secretion). Similar patterns of effects were seen when a higher dose of haloperidol (2.5 mg/kg) was administered for up to 22 days to male rats or to intact or ovariectomized female rats. (Supported by USPHS grants NS15911 and AG2644.)

96.13 EVIDENCE THAT NOREPINEPHRINE DOES NOT MEDIATE THE PERSISTENT EFFECTS OF AMPHETAMINE ON CEREBELLAR PURKINJE NEURONS. S. Sorensen, S. Hattox, S. Johnson*, P. Bickford, R. Murphy,* and R. Freedman* Dept. of Pharmacology, Univ. of Colo. Hlth. Sci. Ctr., Denver, CO 80262

Previous work has demonstrated that chronic amphetamine (Am) administration causes a dramatic slowing in the rate of discharge of Purkinje (P) neurons which persists for months after withdrawal of the Am. Preliminary results suggested that these long-term effects might be mediated by noradrenergic (NE) mechanisms. In these studies we investigated further the role of NE on the P neuron slowing seen after chronic Am. (2 mg/kg i.p. daily for 21 days). Although disruption of the NE input from the locus coeruleus with propranolol, clonidine, or reserpine, partially restored rates to control values, complete recovery to control levels was not observed. Treatment with 6-hydroxydopamine (6-OHDA) following chronic Am did not restore rates to control values either. The results indicate that NE does not mediate the expression of the chronic slowing effect of Am on P neurons. To determine whether NE was involved in the development of this phenomenon, rats were pretreated with 6-OHDA prior to the chronic Am administration protocol. P neurons in these animals were also significantly slowed by chronic Am inspite of a complete lack of NE input throughout the administration protocol. As an additional test of NE function in the chronically treated animals, 3-methoxy-4-hydroxy-phenethyleneglycol (MHPG) concentrations were determined by mass spectrometry. The results indicate that NE does not mediate the chronic effects of amphetamine on Purkinje neuron discharge rate. Other potential mechanisms will be discussed.



96.14 CONTINUOUS HALOPERIDOL IN RATS: A MODEL OF TARDIVE DYSKINESIA; William W. Sant III* and Gaylord Ellison. Dept. Psychol., UCLA, Los Angeles, Ca. 90024.

Tardive dyskinesia (TD) is a neurological disorder characterized by involuntary movements of the mouth, tongue, and facial musculature in individuals who have been administered neuroleptics for an extended period of time, generally at least three months and frequently longer. Some clinicians have proposed that the use of drug holidays, periodic drug-free intervals, is likely to decrease the incidence of TD. Clinical studies investigating the role of drug holidays in TD have been inconclusive, due to a confounding of the number of drug holidays with amount of exposure to neuroleptics. We attempted to investigate this question directly.

We have developed a new model, in rats, of TD. Rats were implanted subcutaneously with slow-release silicone pellets containing a haloperidol solution, and a substantial increase in oral movements was observed when these pellets were removed. These pellets released approximately 0.08 mg of haloperidol per day over a three-week period in 300 gm female, Sprague-Dawley rats. Rats were exposed to haloperidol for 14 weeks using two regimens of haloperidol administration. One group was administered haloperidol without interruption for 14 weeks while another group received three one-week drug-free intervals interspersed among 14 weeks exposure to haloperidol. Both groups demonstrated a TD-like syndrome following discontinuation of haloperidol administration. This syndrome consisted of increased oral movements (rythmic chewing movements and lateral jaw movements) and tongue protrusions. In both haloperidol groups the TD-like syndrome was temporary, lasting approximately three weeks. The time-course of the syndrome differed between these two groups, however. The syndrome developed more rapidly in animals given drug holidays, but was present later after discontinuation of haloperidol administration in animals receiving no such drug-free intervals. These results do not suggest that drug holidays decrease the incidence of TD. Furthermore, we suggest that slow-release haloperidol administration may constitute a useful model, in rats of TD.

(Supported by DA 02969)

96.15 ERYTHROSIN B (FD+C#3) CAUSES INCREASED EXCITABILITY IN A NERVE-MUSCLE PREPARATION. S.L. Cohen*, M.B. O'Neil*, and E. Marder (SPON: H.T. Epstein). Biology Dept., Brandeis University, Waltham, MA 02254.

Erythrosin B (FD+C Red Dye #3) is commonly used to alter the appearance of foods, drugs, and cosmetics. Erythrosin B (Ery) has been reported by others to have a wide variety of effects on voltage-dependent conductances, transmitter release and uptake, and enzyme activities. We applied low concentrations of Ery to an isolated nerve-muscle preparation from the crab, Cancer irroratus and found that Ery applications produced dramatic and irreversible alterations in the properites of the neuromuscular system.

The gm 1 muscles (Marder & Paupardin-Tritsch, J Exp Biol, 88: 147-159, 1980) with nerve attached were placed in a small dish. A stimulating suction electrode was applied to the nerve, and one or two intracellular electrodes placed in a muscle fiber. Under control conditions a train of stimuli to the nerve result in a train of rapidly facilitating Excitatory Junctional Potentials (EJPs). After Ery treatment (10^{-7} to 10^{-5}M for 2-15 min with dissecting lamp on) single stimuli to the nerve often produced long and rapid barrages of EJPs, associated with repetitive firing in the motor axons. Additionally, substantial increases (2-10 fold) in the amplitude of the EJPs were recorded after Ery treatment. The EJP enhancement was accompanied by changes in the rate of facilitation. Ery decreased muscle fiber input impedance slightly, and had little or no effect on postsynaptic ACh responses recorded in voltage clamp. These data strongly suggest Ery has a major presynaptic action.

Ery applications irreversibly stained the nerve pink to deep red. The physiological effects were correlated with the visual observations of staining, and did not occur until after the preparation was exposed to light.

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96.16 EFFECTS OF CHRONIC METHAMPHETAMINE TREATMENT ON APOMORPHINE-STEREOTYPY AND ^{3}H-HALOPERIDOL BINDING IN RATS E. M. Schulz, J. W. Wright and J. W. Harding. Depts. of Psychology and Veterinary Comparative Anatomy, Physiology and Pharmacology, Washington State University, Pullman 99164; RER & D Center, Hines V. A.; MESA Program, University of Chicago

Experimental groups of eight Sprague-Dawley rats received 5 or 10 mg/kg methamphetamine twice-daily for 2, 10, or 20 days. Control groups were maintained in their home cages for equal durations. Sniffing and licking were recorded for one minute at 8, 16, 30, 45 and 60 minutes postinjection of 0.3 mg/kg apomorphine, a dopamine receptor agonist, before and after treatments. The specific binding of ^{3}H-haloperidol (^{3}H-HP), a dopamine receptor antagonist, to corpus striatal tissues was determined following treatments. 1.0 micromolar racemic butaclamol was used to displace saturable binding of ^{3}H-HP. Tissues were recovered by centrifugation. High affinity binding kinetics were estimated with 0.2 to 6.0 nM ^{3}H-HP. Low affinity binding was estimated with 15 and 25 nM ^{3}H-HP. The measure of low affinity binding included high affinity binding.

Methamphetamine treatment (20 days) reduced the estimated density of high affinity sites for ^{3}H-HP by 28% (18 vs 25 pmol/gm-wwt; $p<.06$) compared to matched controls, but did not significantly change receptor affinity (Exp: K_a = 0.5 nM; Cont: K_a = 0.4 nM). In contrast, methamphetamine treatment increased low (plus high) affinity binding 20% (25 vs 21 pmol/gm-wwt; $p<.05$) by 10 days and 60% (34 vs 21 pmol/gm-wwt; $p<.02$) by 20 days over matched controls.

Methamphetamine treatment decreased apomorphine induced licking (from 7 to 2 sec/60 seconds; $p<.01$). This effect was greater with longer durations of treatment. Control animals showed increased licking (from 7 to 10 sec/60 seconds; $p<.01$). Methamphetamine treatment increased the intensity of apomorphine induced sniffing through the first 16 minutes postinjection (from 44 to 53 sec/60 seconds, $p<.01$; posttreatment control: 40 sec/60 seconds) but reduced its duration (from 45 minutes to 30 minutes; $p<.01$).

Our findings provide a basis for earlier noted distinctions between sniffing and licking in animal models using amphetamine (Eichler, Antaluran and Black, Psychopharm., 68, 1980). Continued methamphetamine intoxication may increase apomorphine induced sniffing behavior (early postinjection) by increasing dopamine receptors that have low affinity for haloperidol. The decrease in high affinity receptors for dopamine antagonists found in a previous study on amphetamine intoxication in rats (Howlett and Nahorski, Brain Res., 161, 1979), and in the present study, may underlie the accompanying decrease in apomorphine induced licking.

96.17 CONDITIONED TASTE AVERSIONS TO SLOW ONSET TOXINS: A BEHAVIORAL INDEX OF TOXICITY. A. Riley*, J. Mastropaolo*, and J. Pfaus* (SPON: B. Slotnick) Psychopharmacology Laboratory, The American University, Washington, D.C. 20016

While rats rapidly acquire a conditioned taste aversion to a solution previously paired with a toxin, even when there is a delay of several hours between ingestion and the administration of the toxin, as this delay is increased, i.e., up to and beyond 10 hours, aversions are weak or not evident (Revusky, J. comp. physiol. Psychol. 65: 17-22, 1968). The failure of rats to bridge such taste-toxin delays would clearly be problematic for the use of conditioned aversions as a first tier toxicological screen for slow onset toxins (Parker, Hutchison, & Riley, Neurobehav. Toxicol. Teratol. 4: 93-98, 1982). While the toxin may be administered immediately following access to the taste solution, the rat would be unable to learn an association between the taste and the toxic effects of the drug because of the delay imposed by the slow onset of the drug's toxicity. One way of circumventing this problem of slow onset toxins would be to utilize the non-deprived rat's strategy of drinking in multiple bouts over a night (see Bolles, Theory of Motivation, 1967), a strategy that would insure temporal contiguity between tasting the solution and the onset of the drug's toxic effects. The following experiment tested whether aversions could be acquired in the nondeprived rat when the effects of the toxin were delayed.

Consumption of water from two fluid supplies was automatically recorded during the rats' night cycle (8 PM-8 AM) for 25 consecutive days. After consumption had stabilized, saccharin was placed in the animal's preferred drinking location during the 12 hour drinking period. For four subjects, LiCl was administered 10 hours following the presentation of the saccharin solution. For four control subjects, distilled water injections were given instead of LiCl. This procedure was repeated every fourth day until five pairings had been given.

All subjects showed a marked preference for saccharin on its initial 12 hour exposure, with both groups of subjects displaying drinking bouts in the period preceding drug or control injections. While control subjects maintained this preference for saccharin over repeated tests, poisoned subjects totally avoided the saccharin solution by the fifth saccharin-LiCl pairing.

These data suggest that while slow onset toxins may not induce aversions within the typical taste aversion design, a modification of the design utilizing nondeprived subjects may be effective in detecting toxicity of compounds whose effects are delayed or slow in onset.

96.18 INTERACTION OF FATIGUE STRESS AND CARBON MONOXIDE ON SCHEDULE PERFORMANCE IN RATS. P.S. McGuire and M.M. Preache*. Life Sciences Research Division, IIT Research Institute, Chicago, IL 60616

Exposure to chemical agents rarely occurs in an isolated environment and most frequently a number of other variables are impinging on the organism concurrently. The combination of toxic agent and environmental variables may result in an altered behavioral response to the toxin. To assess the effects of carbon monoxide (CO) when combined with physical stress, rats, food-deprived to 80-85% of their initial body weight, were trained on a chained two-lever variable ratio, fixed ratio schedule for food reinforcement. This schedule required the animal to respond on one lever on a variable ratio 5 (VR 5) for presentation of a light and the opportunity to respond on a second lever on a fixed ratio 15 (FR 15) for food reinforcement. When performance on this schedule had stabilized, animals were randomly assigned to one of four exposure conditions: 0 ppm, 200 ppm, 700 ppm, or 1250 ppm CO. CO exposures were conducted once/week for five consecutive weeks to assess the possibility of tolerance under this exposure regimen. The sixth week, the animals were exposed to the same concentrations of CO but exposures took place following a period of forced swimming. Forced swimming involved weighting the animals with 10 g weights, placing them in 98° water and swimming them until the animals were no longer able to continue swimming or for a maximum of 20 minutes.

The dependent variables analyzed included: responses on the VR lever, responses on the FR lever for food, number of reinforcers obtained and number of light presentations. Data were expressed as percent baseline. Only 1250 ppm CO affected performance by any of the criteria used. At this concentration performance was consistently decreased to 40-50% of baseline in each of the initial five exposure periods. When the rats were swum prior to the sixth exposure session, performance was disrupted in all groups including the 0 ppm group (29-37% of baseline) but the greatest deviation was in the group exposed to 1250 ppm CO (10% baseline). These data suggest that combined exposure to 1250 ppm CO and swimming produce a greater decrement in performance then was observed for either condition alone. (Supported by U.S. Army Contract DAMD 17-80-C-0182.)

97.1 THE EFFECTS OF CHRONIC TRICYCLIC ANTIDEPRESSANT TREATMENT ON ANOREXIA MEDIATED BY PRESYNAPTIC DOPAMINE RECEPTORS.
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Two recent studies have reported that the sensitivity of presynaptic dopamine (DA) receptors is decreased during withdrawal from subchronic treatment with tricyclic antidepressants (TCADs) (Serra, G. et al., Life Sci., 25: 415, 1979; Chiodo, L.A. and Antelman, S.M., Europ. J. Pharmacol., 64: 203, 1980); a third study failed to confirm this finding (Spyraki, C. and Fibiger, H.C., Europ. J. Pharmacol., 74: 195, 1981). We have attempted to assay the sensitivity of presynaptic DA receptors by means of the anorexic response to a low dose of apomorphine (0.06 mg/kg, s.c.). Male rats, maintained on 17-20 hours food deprivation, were allowed to feed for 30 minutes from the pellet dispenser in an operant chamber. The anorexic response to apomorphine was tested at 6-day intervals during 32 days treatment with the TCAD desmethylimipramine (DMI) (7.5 mg/kg, i.p.), and at 3-day intervals during 10 days of withdrawal. The results were subjected to microstructural analysis, based on log survivor analysis of the inter-response time frequency distribution (Willner, P. and Towell, A., Pharmac. Biochem. Behav., in press).

Apomorphine anorexia was characterized by a decrease in both eating rate and eating time; the reduction in eating time was brought about by a decrease in the length of eating bouts, with no consistent effect on intervals between bouts. Changes in eating rate were unaffected by DMI. However, the change in eating time was significantly enhanced by acute (2 days) DMI treatment, causing an enhanced anorexic effect. Subsequently, during chronic DMI treatment, the effect was reversed: significant attenuation of the decrease in eating time was observed on days 8 and 26 of DMI and day 7 of withdrawal, though not on the other test days.

The results provide some support for the hypothesis that presynaptic DA receptors are desensitized by DMI, and suggest 1. that the effect occurs during maintained DMI treatment, not simply during withdrawal, and 2. that the desensitization may be related to an initial increase in stimulation of presynaptic DA receptors. Additionally, the results suggest that eating rate and eating time may be controlled by different DA pathways, only one of which is sensitive to the effects of DMI.

97.2 MULTIPLE DOSES OF AMPHETAMINE COMBINED WITH LOCOMOTOR EXPERIENCE PRODUCE A MARKED ACCELERATION OF RECOVERY OF LOCOMOTION FOLLOWING MOTOR CORTEX INJURY IN CAT.
D. A. Hovda and D. M. Feeney. Department of Psychology, Univ. of New Mexico, Albuquerque, NM 87131.

We have previously established that catecholaminergic agents can influence recovery of locomotion following brain injury (Feeney, D. M., et al. Neurosci. Abst., 6, 802,1980; 7, 930,1981;Hovda, D. A. and Feeney, D. M. Neurosci. Abst., 7, 930,1981). This paper specifically addresses the effect of d-amphetamine (D-AMP) administration with or without locomotor experience during the period of drug intoxication on recovery of locomotion following motor cortex injury in thirty one cats. The cats underwent unilateral motor cortex ablations after assessing their ability to walk or run on a 3.8 m X 5.1 cm beam. Following surgery the animals' beam-walking ability was retested every other day by two raters (one blind to group membership) beginning with day 4 postinjury and continuing to day 30 postinjury. Subjects' beam-walking ability was also tested on days 35,40,50 and 60 postinjury. Beam walking was studied since it reveals a prolonged deficit after motor cortex injury that is not apparent on a flat surface. Subjects were randomly assigned to four groups. A saline group (SAL) which consisted of 10 animals given multiple saline injections 10,14,18, & 22 days postinjury. A single dose of D-AMP group which consisted of 11 animals who received one dose (5mg /kg, i.p.) of D-AMP on the 10th day postinjury. A multiple D-AMP group (MA) was administered 5 mg/kg (i.p.) of D-AMP 10,14,18, & 22 days postinjury. Following drug administrations, animals were placed on the beam at 1,2 and 3 hr post injection to give the subjects the experience of beam-walking while under drug intoxication. Another group of subjects was given the same beam-walking experience and dosing regimen as the MA group but received their drug injections following beam-walking test sessions. Thus these subjects were not given beam walking experience while under drug intoxication. It was found that a single dose of D-AMP significantly improved the rate of locomotor recovery compared to SAL controls. Additionally, multiple doses of D-AMP were found to significantly accelerate recovery compared to a single dose of D-AMP. If subjects were not allowed to experience beam walking while under D-AMP intoxication, the D-AMP accelerated recovery was initially blocked. However, with continued D-AMP administrations a rate of recovery was obtained matching that of subjects who had received D-AMP and experience under the drug. We conclude that multiple doses of D-AMP combined with experience on the task produces maximal acceleration of recovery and that the cat is a good subject for such investigations because of the prolonged deficit.
Supported by NIH grant MBS RR 08139-08 and NS 13684-03.

97.3 DEVELOPMENT OF TOLERANCE TO REPEATED ADMINISTRATION OF 5-METHOXY-N,N-DIMETHYLTRYPTAMINE IN RATS.
G. F. Keltch*, C. D. Himmel* and M. E. Trulson (SPON: A Rupert). Lab. for Neurobiology, Univ.of Texas at Dallas, Richardson, TX 75080.

5-Methoxy-N,N-dimethyltryptamine (5MeODMT), a potent, short-acting hallucinogen in humans, is one of several compounds that are of special interest because of the possibility that they may be synthesized in vivo and act as an endogenous psychotogen. Previous studies have reported that there is no development of tolerance to repeated administration of 5MeODMT, an important feature of a putative psychotogen since the course of psychoses is usually continuous rather than episodic. However, due to the very short half-life of 5MeODMT, we administered the drug at frequent intervals, to determine whether tolerance develops when the drug is constantly present within the body. 5MeODMT produces a "serotonin syndrome" consisting of tremor, rigidity, hindlimb abduction, Straub tail, lateral head weaving and reciprocal forepaw treading in rats. We examined the syndrome-inducing effects of 5MeODMT following chronic administration of the drug (2.0 mg/kg, i.p., every 30 min for 4 h), investigating both the dose-response relationships and the duration of the syndrome following each dose of 5MeODMT (in separate groups). In naive rats, the ED50 for the syndrome-inducing effects of 5MeO-DMT was 1.3 mg/kg, and the mean duration following a 2.0 mg/kg dose was 14.9 min; whereas, after 8 consecutive 5MeODMT injections the ED50 was significantly increased to 2.4 mg/kg and the mean duration following a 2.0 mg/kg dose was significantly decreased to 1.2 min. Rats that received 7 injections of saline separated by 30 min and then various doses of 5MeODMT did not differ significantly from naive rats (ED50 = 1.2 mg/kg; mean duration following a 2.0 mg/kg dose = 16.1 min). Naive rats that received a monoamine oxidase inhibitor (nialamide) showed the syndrome continuously for at least 4 h. When rats were given 5MeODMT chronically and then tested at various time intervals after the final injection, the tolerance had completely disappeared within 4 h following the last injection (ED50 = 1.4 mg/kg; mean duration following a 2.0 mg/kg dose = 13.6 min). Receptor binding studies revealed that chronic 5MeODMT, on the pretreatment regimen described above, resulted in a significant decrease in the B_{max} of ^{3}H-serotonin binding in both the forebrain (-23.9%) and brainstem plus spinal cord (-29.2%) from saline injected control values. Serotonin receptor binding had returned to normal within 4 h following drug withdrawal. Changes within the CNS appear to account totally for the observed tolerance, since the uptake of 5MeODMT into the brain was not altered during the tolerance condition. The present data demonstrate that a profound and rapidly occurring tolerance develops following repeated administration of 5MeODMT, but only if the drug is administered frequently. Therefore, 5MeODMT is not a strong candidate for an endogenous hallucinogen mediating the effects of prolonged psychosis; however, this compound may play a role in acute psychotic episodes.

97.4 BEHAVIORAL EVIDENCE FOR DOPAMINE AUTORECEPTOR SUBSENSITIVITY AND REBOUND SUPERSENSITIVITY:MULTIPLE VS. SINGLE PRETREATMENTS WITH N-n-PROPYLNORAPOMORPHINE.
Richard E. Wilcox, William H. Riffee, and Robert V. Smith
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Previous studies from our laboratories have shown a behavioral supersensitivity to the stereotypic effects of apomorphine following chronic administration of the dopamine agonists apomorphine (APO) and N-n-propylnorapomorphine (NPA). Parallel studies of changes in striatal (3H)-spiroperidol receptor binding and in vivo dopamine turnover have suggested that a subsensitivity of striatal dopamine autoreceptors plays a significant role in the behavioral supersensitivity to APO challenge after chronic dopamine agonist administration.

We have evaluated the effects of single pretreatments with NPA and APO on the stereotypic activity induced by subsequent APO challenge doses 1, 3 and 5 days later. Time-and dose-response analyses of videotaped behavior show a consistent subsensitivity in comparison to the response to APO challenge after saline pretreatment. In contrast, a single pretreatment with haloperidol (4 mg/kg, i.p.) is followed (72 hours later) by a supersensitivity to challenge doses of APO. We suggest that a single aporphine pretreatment may produce a subsensitivity of striatal dopamine autoreceptors which is rapidly followed by a longer listing rebound autoreceptor supersensitivity (manifest as a behavioral subsensitivity). If multiple aporphine pretreatments are administered the period of autoreceptor subsensitivity following each injection might be progressively increased in duration (manifest as a supersensitive behavioral response) after 14 aporphine pretreatments.

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97.5 REDUCED ABILITY OF LOW DOSES OF APOMORPHINE TO INHIBIT DOPAMINE RELEASE AFTER CHRONIC APOMORPHINE. MODIFICATION OF DEXTROAMPHETAMINE INDUCED LOCOMOTOR ACTIVITY.

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Low doses of apomorphine (APO) can inhibit dopamine turnover and release, in part, via an action on presynaptic (perhaps axon terminal autoreceptor) mechanisms. We have previously found that chronic administration of APO produces a behavioral supersensitivity to the stereotypic effects of APO challenge, in part, through a striatal dopamine autoreceptor subsensitivity. The locomotor effects of dextroamphetamine (AMP) appear to involve nonstriatal and, perhaps striatal, presynaptic adrenergic and dopaminergic mechanisms. The purpose of this study was to determine the effects of chronic APO administration on the dopaminergic component of these mechanisms.

APO in low doses inhibits dextroamphetamine-induced locomotor activity in mice. Changes in the locomotor response (horizontal activity measured via Omnitex infrared sensors) to a challenge dose of AMP (2.5 or 5 mg/kg, i.p.plus various doses of APO from 0.019-0.300 mg/kg were determined after chronic saline or APO administration. After chronic saline, AMP-induced locomotor activity was inhibited by APO with 0.300mg/kg APO reducing AMP-activity to saline control levels. Following chronic APO administration, the ability of APO to inhibit AMP-induced locomotor activity was still present but at a reduced level. In contrast, the effects of AMP alone were unchanged by chronic APO treatment. These data are consistent with the suggestion that chronic APO administration alters the ability of APO to modulate the synthesis and/or release of dopamine presynaptically. The effects of AMP to enhance the synaptic content of dopamine are not similarly altered. Therefore, since the presynaptic actions of APO, but not AMP, are mediated, in part, by axon terminal autoreceptors, these autoreceptors may play a role in the altered response to APO which occurs after chronic APO treatment. (Supported in part by MH-33442 to WHR and REW.)

97.6 POSSIBLE DOPAMINE ANTAGONIST-LIKE EFFECTS OF S-(+)-APOMORPHINE.

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The prototype dopamine agonist antiparkinson drug is R-(-)-apomorphine (R-APO). Studies from our laboratories have suggested, contrary to previous reports, that S-(+)-apomorphine (S+APO) may have antagonist-like properties in vivo and in vitro. Our basic findings with S+APO may be summarized as follows.

1. TD50 and LD50 are similar for S+APO and R-APO. LD50 100 mg/kg ip for S+APO and equals 128 mg/kg ip for R-APO in mice.
2. Behaviorally, S+APO is an effective antagonist of the stereotypic effects of R-APO in mice.
3. Inhibition of striatal (3H)-spiroperidol, (3H)-apomorphine and (3H)-N-n-propylnorapomorphine receptor binding in vitro by S+APO and R-APO indicates that S+APO is about one-third as potent as R-APO in competing for neuroleptic binding sites and about one-twelfth as potent in competing for aporphine binding sites in calf, rat and mouse.
4. In subtoxic doses, S+APO is devoid of obvious behavioral signs of dopamine agonist activity (mice) and is unable to stimulate striatal adenylate cyclase activity (rat).
5. In vivo dopamine turnover in the striatum may be detected via l-dopa vs. dopamine levels after decarboxylase inhibition using HPLC (reverse phase with electrochemical detection). Using this protocol R-APO administration produces dose-dependent decreases in dopamine turnover in mice. In contrast S+APO injection results in dose-dependent increases in dopamine turnover.

We suggest that S+APO may have only a very weak interaction with an active site on a dopamine receptor. However, S+APO may be quite effective in occupying allosteric sites on the same receptor resulting in an effective blockade of these sites. According to this view, S+APO administration would lower the probability for endogenous dopamine or R-APO interacting with the allosteric dopamine receptor sites. An antagonist-like effect of S+APO could result because the allosteric site blockade could render the fit of true agonists with an active site less perfect. (Supported in part by NS-12259 to RVS, MH-33442 to WHR and REW and UT-BRSG to REW.)

97.7 BEHAVIORAL EFFECTS OF SEROTONERGIC AND DOPAMINERGIC DRUGS IN CATS FOLLOWING CHRONIC AMPHETAMINE ADMINISTRATION.

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Chronic (but not acute) administration of amphetamine to cats elicits a number of behaviors, such as limb flicking, abortive grooming, head shakes, and investigatory and hallucinatory-like responses, which were originally proposed as an animal behavioral model for studying the actions of hallucinogens that depress central serotonergic neurotransmission (Jacobs, et al., Brain Res. 132, 1977, 301-314). Subsequent neurochemical studies revealed that chronic amphetamine treatment decreased the central metabolism of both serotonin and dopamine (Trulson and Jacobs, J. Pharm. Exp. Ther. 211, 1979, 375-384). Recently, it was reported that apomorphine, a potent dopamine agonist which does not depress central serotonin metabolism, also elicits these behaviors in cats (White, et al., Pharm.Biochem. Behav.14, 1981, 339; Trulson and Crisp, Eur. J. Pharm. in press). The present study was designed to investigate the relative importance of dopamine and serotonin in mediating these behavioral effects in cats following chronic amphetamine treatment. Since the limb flick (LF) has been shown to be the best behavioral index in this model, it was used for the quantitative analysis in the present study. Groups of adult male and female cats were administered d-amphetamine sulfate (10 mg/kg, i.p.) every 12 h for 10 consecutive days. In agreement with previous studies, cats displayed a mean LF rate of 13.8/h (vs 0.2/h on saline baseline) following this treatment regimen. Administration of L-tryptophan (20 mg/kg, i.p.), L-5-hydroxytryptophan (10 mg/kg, i.p.) or a monoamine oxidase inhibitor (tranylcypromine, 4 mg/kg, i.p.) produced no significant change in the LF rate following chronic amphetamine treatment. However, administration of L-DOPA (10 mg/kg, i.p.) plus a peripheral decarboxylase inhibitor (MK486, 50 mg/kg, i.p.) greatly potentiated the behavioral response in cats treated chronically with amphetamine (29.2 LF/h). L-DOPA in combination with a peripheral decarboxylase inhibitor produced no LF in naive cats. The effects of apomorphine (2 mg/kg, i.p.) were also significantly potentiated by chronic amphetamine treatment (22.4 vs 2.5 LF/h in naive cats). Neurochemical analysis revealed that brain serotonin was depleted by 44.9%, while striatal dopamine was depleted by 96.2% following chronic amphetamine treatment. These data suggest that the LF response is elicited by an action on central dopamine receptors, and that these receptors become supersensitive following chronic amphetamine administration. Furthermore, there may be a qualitative change in the receptors, since L-DOPA is very effective in potentiating these behaviors in cats treated chronically with amphetamine, but is totally ineffective in naive cats.

97.8 STRESS-INDUCED ALTERATIONS OF NOREPINEPHRINE: CROSS-STRESSOR SENSITIZATION.

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A series of experiments were designed to evaluate the effects of uncontrollable stressors on alterations of brain norepinephrine (NE) in several brain regions. Exposure to inescapable footshock provoked a transient reduction of NE in hypothalamus, and to a lesser extent in locus coeruleus, hippocampus and cortex. The reduction was longer lasting after 180 than after 60 shocks (6 sec duration, 150 uA). Moreover, the extent of the reduction was found to vary as a function of the time of day, being more pronounced when animals were stressed and sacrificed between 0830-1000 and 1300-1430 hr than between 1700-1900 hr. When animals were reexposed to the stressor 24 hr later the amine reduction was readily reestablished, even when the severity of the second stress was reduced. Indeed, the enhanced NE reductions were evident among mice that had previously received exposure to a different form of stress (e.g., cold water immersion). In effect, the exaggerated neurochemical effects of stress were evident in a cross-stressor paradigm. In studies where mice received a session of shock stress each day for 15 consecutive days, the NE reductions ordinarily provoked by acute stress were absent; however, the increased NE utilization induced by shock reexposure was still evident. Together, these data suggest that the mechanisms subserving the alterations of NE induced by a stressor are subject to sensitization. With chronic stress application the increases of NE concentrations are likely due to a compensatory increase of synthesis, but without a reduction in the rate of amine utilization. Data are discussed with respect to the behavioral alterations induced by controllable and uncontrollable stressors.

97.9 BEHAVIORAL ALTERATIONS PRODUCED BY LONG-TERM TREATMENT WITH DOPAMINE AGONISTS: APOMORPHINE AND BROMOCRIPTINE. Connie B. Grogan* and George V. Rebec (SPON: R. M. Wightman). Dept. of Psychol., Indiana Univ., Bloomington, IN 47405.

A key feature of the schizophreniform psychosis produced by dopamine (DA) agonists is its gradual development with multiple injections. Thus, an animal model of this disorder should include only those behaviors that are enhanced or sensitized during long-term treatment with these drugs. A large body of research with amphetamine indicates that in rats repeated injections selectively enhance sniffing and repetitive head movements, whereas tolerance develops to licking and biting (oral behaviors). To determine if this selective augmentation of behavior is common to other DA agonists and thus comprises a viable model of drug-induced psychosis, we examined the behavioral alterations in rats produced by long-term treatment with apomorphine and bromocriptine.

Adult, male rats received a single, daily injection of saline, 1.0 mg/kg apomorphine, or 10.0 mg/kg bromocriptine for 14 consecutive days. Individual items of behavior (e.g., forward locomotion, rearing, sniffing, head bobbing, licking, and biting) were recorded on the 1st, 8th, and 14th day of treatment. Our analysis revealed that, like amphetamine, both apomorphine and bromocriptine selectively increased sniffing and repetitive head movements with repeated injections. These behaviors were also enhanced in both groups following a challenge injection of 0.5 mg/kg apomorphine at various times after cessation of the chronic treatment schedule. Moreover, apomorphine-induced oral behaviors were reduced in both the apomorphine and bromocriptine pretreated animals compared to saline controls. Thus, long-term treatment with these DA agonists produced behavioral changes in the rat that paralleled those produced by amphetamine. These results, which argue against a general augmentation of behavior with repeated injections of DA agonists, support the view that only a limited number of behavioral responses can serve as an animal model of the psychosis produced by these drugs.

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97.10 PERMANENT ATTENUATION OF SEPTAL LESION IRRITABILITY BY APOMORPHINE MICROINJECTIONS INTO THE OLFACTORY TUBERCLE. R.F. Marotta*, N. Gubitosa*, J. Kristofech*, E.L. Gardner, and H. Weiner. Dept. of Psychiatry, Montefiore Hospital and Albert Einstein College of Medicine, Bronx, New York.

Although numerous mechanisms have been proposed as underlying recovery from damage to the central nervous system, the actual processes involved in specific instances are little understood. A major problem has been the difficulty in clearly delineating the relationship of structures distant to the site of lesion, to the ultimate dissipation of behavioral effects. Once a functional circuit important to recovery in a specific syndrome is found, mechanisms on a more molecular level may be investigated. We previously reported (Marotta et al., Nature 264:513, 1977) that systemic administration of dopamine agonists greatly accelerate the rate of recovery from the septal lesion induced irritability syndrome (SIS). Treated rats reached preoperative baseline levels of irritability within 3 hrs. of drug administration, while controls did not reach that level for at least 7 days. Here we present evidence based on intracerebral microinjection of apomorphine (Ap) that the anterior ventral brain region around the olfactory tubercle (OT) is the critical telencephalic structure in recovery from the SIS in rodents.

Individually housed, ad lib fed and watered, male Sprague-Dawley male rats weighing 320 gm were rated for irritability using a modified King scale (0 to 3 points for: biting, reaction to probe, height jumped, difficulty in capture and handling, vocalization, and reaction to air puff) for three preoperative days. Using standard stereotaxic technique four surgical groups were prepared under Brevital anesthesia: 1. OT cannula-septal lesion (SL); 2. caudate nucleus cannula-SL; 3. amygdala cannula-SL; 4. OT cannula-nonlesion control. All placements and lesions were histologically verified. 24 hr. post-surgery rats were rated for the SIS by a trained observer blind as to treatment. Animals were then pair-matched on the basis of SIS scores. OT cannula SL rats were microinjected with either Ap (10 ug/5 ul; n=10) or saline-ascorbate vehicle (n=10), and then rated every 30 min. for 3 hrs., and once a day for 10 days. Treated animals reached SIS baseline levels within 24 hrs., while controls did not do so for 6 days. An equal amount of Ap into either the caudate or amygdala (n=6) did not significantly decrease the SIS below controls. Lidocaine (50 ug/5 ul) microinjected into the OT region of recovered SL rats (n=12) resulted in a return of the SIS lasting 40 minutes. However, lidocaine into the OT region of nonlesion control did not result in an increase in irritability. Therefore, the region around the OT seems to be critical in recovery from the SIS, as well as perhaps constituting an important locus for irritability states in general.

97.11 ELECTRICAL STIMULATION-INDUCED ROTATIONAL BEHAVIOR: INVOLVEMENT OF THE NIGROSTRIATAL DOPAMINE SYSTEM. Edward Castañeda*, Terry E. Robinson and Jill B. Becker (SPON: L. Rutledge). Psych. Dept. and Neuroscience Lab., Univ. of Michigan, Ann Arbor, MI 48109.

Electrical stimulation of ascending nigrostriatal dopamine (DA) neurons causes rats to turn vigorously away from the stimulated side (contraversive), and may thus provide a behavioral index of nigrostriatal DA activity. We have studied whether the nigrostriatal DA system specifically is necessary for contraversive electrical stimulation-induced rotational behavior (ESRB). Holtzman rats were implanted unilaterally with a bipolar electrode into the nigrostriatal bundle as it courses through the hypothalamus and a guide cannula aimed at the substantia nigra. After 2 weeks recovery the rats were tested for ESRB by applying current (0.1 msec duration pulses, 50 pulses/sec) for 10 sec and counting the number of 1/4 turns (90°) made. Each animal was stimulated at 100, 150, 200, and 300 μA each day for 5 days of baseline testing. After testing on the 5th day the rats received either 6-hydroxydopamine (6-OHDA; 8 μg in 4 μl) or 0.9% saline (4 μl) via the chronically implanted cannula. The animals were then tested for ESRB daily for 8 more days. After their initial testing on the 8th day post-6-OHDA or saline all rats were injected i.p. with a low dose of α-methyl-p-tyrosine (MPT; 40 mg/kg) and tested 2, 4, and 6 hrs later. At least 5 days later the rats were killed and forebrain structures assayed for DA. After the 6-OHDA infusion 1 or 2 effects were obvious. Some of the lesioned rats which had shown consistent contraversive ESRB suddenly switched direction and showed vigorous ipsiversive ESRB (switchers). The remaining rats showed a transient decline in contraversive ESRB, followed by an increase in ESRB above baseline levels (nonswitchers). Analysis of striatal DA content revealed that all the rats which switched direction had >96% DA depletions ($\bar{X}$=98.2). The nonswitchers had <95% striatal DA depletions ($\bar{X}$=68.1). There was no overlap between the 2 groups in the magnitude of DA depletion. The control rats showed no change in ESRB. In the nonswitchers MPT treatment significantly attenuated contraversive ESRB. However, in the saline-treated control animals and the switchers MPT failed to change ESRB. We suggest that contraversive ESRB is dependent on an intact nigrostriatal DA system. However, to disrupt contraversive ESRB this sytem must be nearly totally destroyed. The switch in turning direction seen when >95% DA depletions were achieved may be due to unmasking the effects of current spread to another, more distant, neural system (e.g. fibers in the crus cerebri). It is interesting that nonswitchers showed heightened ESRB after 6-OHDA. It is possible that an incomplete lesion results in a compensatory response in the lesioned side producing hyperactivity in the remaining neurons. (Supported by NS16437)

97.12 PLASTICITY IN STRIATAL DOPAMINE ACTIVITY: BEHAVIORAL AND BIOCHEMICAL EVIDENCE. Jill B. Becker, Terry E. Robinson and Sharon K. Presty*. Psychology Department and Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

Amphetamine (AMPH)-induced rotational behavior in non-lesioned rats and AMPH-stimulated dopamine (DA) release from striatal tissue fragments in vitro were used to study the long-term effects of a single injection of AMPH on activity in the mesostriatal DA system. In the behavioral tests, intact rats were injected with AMPH and placed in automated rotometers for 1 hr where the following were recorded: (1) 1/4 (90°) turns; (2) Rotations (360° turns) and (3) Net Rotations (rotations in the dominant direction minus those in the other direction). A single injection of a low dose of AMPH (1.25 mg/kg) greatly enhanced the rotational behavior produced by a second injection of AMPH given 3-4 weeks later in intact female, ovariectomized female and castrated male rats. Animals in these groups made 50-60 more net rotations (average increase of 105%) during the 2nd test session than during the 1st. The effect of AMPH pretreatment in intact males differed from that in the other groups. The intact males showed enhanced rotational behavior only during the first 30 min of the 2nd test session. During the 2nd 30 min of the 2nd test session these males actually turned less than they did during the 1st. When only 7-8 days separated the two test sessions intact male and female rats both showed sensitization of rotational behavior, but the magnitude of the change was greater in females.

We also studied the effect of AMPH pretreatment on striatal DA release using an in vitro continuous flow perifusion system. Ovariectomized female rats received either 1.25 mg/kg AMPH (n=8) or saline (n=8) and were then returned to their home cages for 3-5 weeks. Rats were then killed; striatal tissue was removed and placed in a perifusion chamber. After obtaining a basal DA release rate, DA release in response to a pulse of AMPH was determined. Striatal tissue from rats which had been previously exposed to AMPH 3-5 weeks earlier showed significantly greater AMPH-stimulated DA release than did striatal tissue from saline injected controls.

We conclude that: (1) repeated injections of AMPH are not necessary to produce a long-lasting facilitation of behaviors mediated by the mesostriatal DA system; (2) gender and/or hormonal state influences the development of long-term changes in the mesostriatal DA system; and (3) changes in DA release from presynaptic terminals may contribute to the behavioral sensitization produced by stimulant drugs. The phenomena reported here may provide complementary in vitro and in vivo models for studying neuroplasticity in brain DA systems.

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97.13 PROTEIN SYNTHESIS IN RATS TREATED CHRONICALLY WITH AMPHETAMINE. M. M. Widelitz, C. P. Hable II* and N. G. Avadhani*. VA Medical Center, Coatesville, PA 19320.

It is well established that amphetamine given in a single dose can induce a stereotypic behavior pattern in rats which has been equated to paranoid schizophrenia in humans. It has also been shown that a single injection of amphetamine can cause disaggregation of polyribosomes in vivo and inhibit protein synthesis in vitro. We have recently utilized amphetamine chronically, injecting animals with amphetamine over a thirty-day period. While one induces a progressive augmentation of locomotor activity and stereotypies as well as disaggregation of polyribosomes, surprisingly we do not see the inhibition of protein synthesis. In some animals, we have actually seen stimulation. The possible reasons for this will be discussed. Supported by U.S. Veterans Administration, Washington, D.C.

97.14 TOLERANCE TO AMPHETAMINE-INDUCED INHIBITION OF NEURONAL ACTIVITY IN THE CENTRAL AMYGDALOID NUCLEUS. George V. Rebec and Eunjee H. Lee. Dept. of Psychol., Indiana Univ., Bloomington, IN 47405.

We have previously shown that long-term administration of d-amphetamine in the rat produced a progressive augmentation of the effects of this drug on neuronal activity in the neostriatum (Rebec and Groves, Pharmac. Biochem. Behav., 1976, 5:349; Alloway and Rebec, Soc. Neurosci. Abstr., 1981, 7:266). This change may mediate, at least in part, the behavioral augmentation that accompanies multiple amphetamine injections (Rebec and Segal, Pharmac. Biochem. Behav., 1980, 13:793). Interestingly, however, not all components of the amphetamine behavioral response are enhanced with long-term treatment suggesting that the neuronal augmentation may not be generalizable to other forebrain sites. The central amygdaloid nucleus (CAN), for example, has been implicated in amphetamine-induced licking and biting which actually decline with repeated injections. In the present series of experiments, therefore, we extended our electrophysiological analysis to neurons in the CAN following acute or long-term amphetamine administration.

Adult, male rats were pretreated twice daily with saline or 2.5 mg/kg d-amphetamine for 5 consecutive days. Approximately 12 hours after the last injection, tungsten microelectrodes were lowered bilaterally into the CAN and the animals were challenged with 0.2 mg/kg d-amphetamine administered intraveneously at 2-minute intervals. Control rats consistently responded with an inhibition of neuronal activity by the fourth injection (cumulative dose: 0.8 mg/kg). Amphetamine-pretreated rats, on the other hand, were significantly less responsive. In fact, the majority of CAN neurons failed to respond even by the tenth injection (cumulative dose: 2.0 mg/kg). It appears, therefore, that tolerance develops to the electrophysiological actions of amphetamine in the CAN but not in the neostriatum. These results suggest a possible neuronal substrate for the differential behavioral effects of long-term amphetamine treatment. Furthermore, because both the CAN and the neostriatum receive afferents from the mesotelencephalic dopamine (DA) system (Moore and Bloom, Ann. Rev. Neurosci., 1978, 1:129), our results indicate that a change in presynaptic DA dynamics cannot completely explain the effects of long-term amphetamine administration on neurons in postsynaptic sites.

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98.1 STRESS-INDUCED NEUROLOGICAL DEFICITS IN DOPAMINE-DEPLETED RATS AND THEIR REVERSAL BY DOPAMINE AGONISTS. Abigail M. Snyder*, Michael J. Zigmond and Edward M. Stricker (SPON: David J. Kupfer). Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Animals sustaining brain dopamine (DA) depletions of at least 90-95% exhibit profound behavioral dysfunctions such as akinesia and catalepsy. These abate with time but can be reinstated when animals are exposed to acute glucoprivation (Stricker et al., J. Comp. Physiol. Psychol. 93:512, 1979). In the present experiments the relationship between DA depletions and stress-induced neurological impairments was shown to be altered by drugs which act at DA synapses, and was extended to include the response to a variety of stressors.

Selective DA depletions were produced in adult rats by intraventricular injections of 6-hydroxydopamine (200 or 250 µg in 20 µl) following pretreatment with desmethylimipramine (25 mg/kg, ip) and in some cases, pargyline (50 mg/kg, ip). Animals with striatal DA depletions of 10-40% did not exhibit neurological impairments when acute glucoprivation was induced by systemic 2-deoxyglucose (2DG, 500 mg/kg, ip), but they did so when pretreated with the DA receptor blocker fluphenazine (0.01 mg/kg, sc). (This dose of fluphenazine alone had no apparent effect on behavior.) As noted previously (Snyder et al., Soc. Neurosci. Abstr. 6:91, 1980), animals with >90% DA depletions exhibited akinesia and catalepsy following 2DG (500 mg/kg). We now report that these impairments were reversed by apomorphine (0.1 mg/kg, sc) or l-dopa (60 mg/kg, ip). Caffeine, and other drugs less selective in their action on dopaminergic neurons, were less effective in this regard. These data suggest that an acute DA deficiency may mediate the production of neurological deficits in brain-damaged rats after 2DG treatment.

In the second series of experiments, animals with variable DA depletions were given each of the following treatments on separate occasions: hypertonic saline injection (5 ml of 1M NaCl, ip), insulin (4 U/kg, sc), tail shock (1 mA shocks every 5 sec for 10 min), or 24-hr food deprivation. Hypertonic saline and insulin treatments each were capable of producing neurological dysfunctions in rats with large DA depletions. Tail shock also was effective in these animals, whereas food deprivation was much less so. These results suggest that in animals recovered from severe DA-depleting lesions, a variety of acute stressors can reinstate neurological deficits.

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98.2 IMPAIRED ACQUISITION OF AN OPERANT RESPONSE IN YOUNG RATS DEPLETED OF BRAIN DOPAMINE IN NEONATAL LIFE. T.G. Heffner and L.S. Seiden Dept. Pharmacol. & Physiol., Univ. of Chicago, Chicago, IL 60637.

In an attempt to determine if brain dopamine (DA) neuron destruction alters learning ability in the developing rat, the rate of acquisition of a positively reinforced lever pressing response was examined in young male and female rats (30-45 days of age)following treatment with desmethylimipramine (DMI, 20 mg/kg,sc) and 6-hydroxydopamine (6-HDA, 35 µg, ivt) at 3 and 6 days of age. Control rats received DMI plus the 6-HDA vehicle solution. The 6-HDA treatment reduced DA by 75% in the caudate, by 54% in the frontal cortex and by 54% in the nucleus accumbens but did not alter brain content of norepinephrine. The 6-HDA-treatment also did not alter growth rate or water intake. Moreover, locomotor activity, which is elevated by this 6-HDA treatment during the first 3-4 weeks of life, was not different from control levels at the time acquisition testing began. Control rats (N=11) and 6-HDA-treated rats(N=17) were deprived of water for 20 hr daily and were exposed for 1 hr daily to a fixed ratio schedule of reinforcement which employed an autoshaping procedure; each depression of a response lever or the passage of 1 min during which no lever press occurred resulted in the presentation of 20 µl of water for 4 sec. Control rats rapidly acquired the operant schedule; 10 of 11 rats achieved the criterion for acquisition (50 reinforced lever presses per hr) after 4 sessions, and all control rats had acquired the schedule after 6 sessions. In contrast, only 8 of the 17 6-HDA-treated rats had acquired the schedule after 8 sessions and 4 rats failed to acquire the schedule after 16 sessions. Control rats required 3.1 ± 0.5 sessions (mean ± SEM) for acquisition whereas the 13 6-HDA-treated rats that acquired the schedule required 7.8 ± 0.7 sessions for acquisition. Although the rate of acquisition did not differ with sex among the control rats, male rats given 6-HDA as neonates required significantly more sessions for acquisition (9.7 ± 1.0 sessions) than female rats given 6-HDA as neonates (6.2 ± 0.6 sessions). Of the 4 6-HDA-treated rats that failed to acquire the schedule, 3 were males. There were no sex-related differences in the extent of brain DA depletion caused by 6-HDA. After the 6-HDA-treated rats had acquired the operant schedule, their performance was indistinguishable from that seen in control rats. These results suggest that neonatal loss of brain DA projections can result in impaired acquisition of an operant response. This effect appears to be unrelated to changes in growth rate, water intake and locomotor activity and is not accompanied by deficits in performance of the response once acquisition has occurred. These results support previous proposals that the DA-depleted neonatal rat represents a model for Attentional Deficit Disorder (Minimal Brain Dysfunction) a disorder more common among male children than female children. (Supported: NS-12324 and MH-10562).

98.3 PLATELET MONOAMINE OXIDASE ACTIVITY AND BEHAVIORAL RATINGS. Patricia Tueting and Herbert Y. Meltzer. Lab. of Biological Psychiatry, Ill. State Psychiatric Institute, and Dept. Psychiatry, University of Chicago, Chicago, Illinois.

In normal subjects, low platelet monoamine oxidase (MAO) activity has been related to stimulus seeking behavior (Donnelly et al., Biol. Psychiat., 1979, 14:375). In psychiatric patients, reports have been conflicting. Auditory hallucinations and paranoia, the syndromes of bipolar disorder and chronic schizophrenia, and poor response to lithium have been reported to be characteristic of low MAO activity. Anxiety and depression are the traits that have been linked to high MAO activity (Matthew et al., Am. J. Psychiat., 1981, 138:371).

Our pharmacological studies of MAO activity indicate that there are probably drug confounds in many of these studies (Meltzer et al., Brit. J. Psychiat., 1982, 140:192). We have also found that another variable which needs to be considered is duration of illness. We typically find a negative correlation between MAO activity and duration of illness in our patient populations. This is in spite of the fact that a positive correlation between MAO activity and age is found in normal subjects (Robinson and Nies, Schiz. Bull., 1980, 6:298). Our strategy in this study was to cope with the above conflicting reports by assessing these potential confounds. Also, since we had a large data base (N=233 inpatients), we used the split half technique to indicate reliability given the large between subject variance typical for MAO activity.

The first analysis was done separate for each sex without regard to diagnosis. Several correlations between platelet MAO and ratings on clinical items were significant. However, in no instance were correlations significant for both data halves. An exception to this trend was found in a second analysis in which patients were grouped into broad diagnostic categories. A trend for low MAO to be associated with traits commonly considered to be typical of mania was found when patients were grouped according to the presence of the manic syndrome.

We also found a trend for low platelet MAO to be associated with poor response (global clinical ratings) to lithium in these manic patients which supports Sullivan et al. (The Lancet, 1977, ii:1325). In apparent contradiction to the finding with global clinical ratings, low MAO was associated with greater improvement in manic items with lithium treatment. The Sullivan et al. finding may be in part due to the fact that patients with both long duration of illness and low platelet MAO are generally less responsive to any form of drug or psychosocial treatment. Supported by USPH 25116, MH30938, and Ill. Dept. Mental Health

98.4 REVERSAL OF BEHAVIORAL DEPRESSION BY INFUSION OF AN ALPHA-2 ADRENERGIC AGONIST INTO THE LOCUS COERULEUS. P. A. Goodman*, J. M. Weiss*, L. J. Hoffman*, M. J. Ambrose*, W. H. Bailey, and J. M. Charry*. Lab. of Behavioral Biology, The Rockefeller Univ., New York, NY 10021.

This experiment demonstrated that behavioral depression produced by uncontrollable shock in rats could be reversed by microinfusion of the alpha-2 adrenergic agonist clonidine into the region of the locus coeruleus (LC). Animals subjected to uncontrollable shock show behavioral depression in a swim test where active behavior (struggling and floating) is quantified. The animals subjected to uncontrollable shock (Shock group) show substantially more floating and less struggling behavior than animals that have not received shock (No-shock group). This shock-induced depression has been found to be highly correlated with large depletions of norepinephrine (NE) in the LC (Weiss, J.M., Goodman, P.A., Losito, B.G., Corrigan, S., Charry, J.M., and Bailey, W.H., Brain Res. Rev., 3:167, 1981). To test the hypothesis that this depletion of NE produces depression through reduced stimulation of alpha-2 receptors in the LC, we investigated whether infusion into the LC of drugs that affect alpha-2 receptors would affect stress-induced behavioral depression.

Animals were subjected to uncontrollable tail-shock on a schedule shown to produce behavioral depression. Following shock and just prior to the 15-minute swim test, animals were infused bilaterally in the region of the LC with clonidine, piperoxane (an alpha-2 adrenergic antagonist), or vehicle for 20 minutes (drug concentration was 0.16 µg/µl and was administered at a rate of 0.1 µl/minute). Shocked animals infused with vehicle exhibited significantly less activity than No-shock animals infused with vehicle; thus, the usual depression of active behavior was seen after shock. Shocked animals infused with piperoxane also showed behavioral depression. In contrast, shocked animals infused with clonidine were significantly more active than both the Shock-vehicle and the Shock-piperoxane animals, and were not significantly different from the No-shock-vehicle animals that were not behaviorally depressed. These results are consistent with other data which show that blockade or stimulation of alpha-2 receptors in the LC of normal animals leads to behavioral depression or excitation, respectively (Weiss, J.M., Bailey, W.H., Goodman, P. A., Hoffman, L.J., Ambrose, M.J., Salman, S., and Charry, J.M., In Behavioral Models and the Analysis of Drug Action, A. Levy and M.Y. Spiegelstein {Eds.}, in press). In conclusion, the depressant effect of uncontrollable shock on behavior was reversed by infusion of clonidine into the LC thus supporting the hypothesis that a deficiency of NE at alpha-2 receptors in the LC is responsible for stress-induced behavioral depression.

98.5 THE EFFECT OF AN ENVIRONMENTAL STRESS, CAGING CONDITIONS, ON EXPERIMENTAL RESULTS IN ADRENAL PNMT STUDIES IN RODENTS.
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Many studies have focused on the genetic differences between strains of mice and rats with respect to the adrenal catecholamine biosynthetic enzymes. In recent studies dealing with adrenal phenylethanolamine N-methyltransferase (PNMT) activity in C57BL/6 and DBA/2 mice, we have found that both the initial stress level of the animals and the housing design used in the experiment can significantly influence what changes in adrenal PNMT levels will occur in response to various treatments. This represents an interaction between environmental factors and treatment responses. We have observed this in each of our strains of mice as well as in Sprague-Dawley rats.

Male mice of each strain were housed either in groups of four mice of the same strain or individually in 6½x11x5 inch plastic cages. Among the group housed mice, significant cage effects were found between cages of identically treated mice. The magnitude of cage effects varied between experiments. On some occasions, the differences between cages correlated with differences in the extent of fighting observed between cages. There was a larger variance among adrenal PNMT activity measurements of group housed animals than individually housed animals. These cage differences were likely a result of the behavioral interactions between animals in the group housed cages.

In experiments using group housing, animals in cages having higher control levels of adrenal PNMT activity produced smaller increases in PNMT activity in response to orchiectomy and ACTH.

In replicate experiments conducted first on mice which were group housed, with each treatment being represented in each cage, and secondly on the same strain which were individually housed, the effect of orchiectomy was much smaller in the second than in the first experiment, indicating that part of the effect of this operation on adrenal PNMT activity could be mediated through the behavioral interactions between the animals. In addition, the effects of the drug and operation, when given simultaneously, were additive (on a log scale) in individually housed animals and were interactive in the group housed animals (e.g., the drug and operation given together produced a larger response than the sum of the treatments given separately).

The finding of cage effects and the housing-treatment interaction were quite consistent throughout these experiments. It can be concluded that there was no single genetic response of each strain to the treatments given. The possibility of differential strain responses to housing conditions may complicate the interpretation of studies dealing with strain differences in variables which are affected by stress. (Funded by NIMH MH 25998 & MH 00219.)

98.6 ROLES FOR CANONICAL NEUROTRANSMITTERS IN REVERSAL OF CONDITIONED EMOTIONAL RESPONSE BY DIAZEPAM, J.D. Lane, J.D. Altazan*, D.R. Cherek and J.E. Smith. Psychiatry Research Unit, LSU Medical Center, Shreveport, LA 71130 USA.

Conditioned emotional response (CER) is thought to be an animal model of anxiety. CER has frequently been used to evaluate behavioral-drug effects, with anxiolytics clearly attenuating the emotional response. To evaluate the neurochemical changes associated with this paradigm and its reversal by diazepam, groups of littermate Fisher F-344 rats were food deprived and shaped to lever press in a standard operant chamber. Lever pressing was maintained by a variable interval (VI) 1 min schedule of food presentation. Following stabilization of responding, one hour classical conditioning sessions were initiated. Classical conditioning consisted of presenting a conditioned stimulus (tone) of varying lengths with an unconditioned stimulus (foot shock). To isolate the conditioning component from the shock history and animal activity, the other two littermates received yoked CS (tone) or USC (shock) only. Additional CER animals received vehicle or 5 mg/kg i.p. diazepam. Radiolabelled precursors were administered at various pulse times to evaluate turnover of biogenic amines and amino acids. On test day, the CS was presented to the groups for 15 minutes while lever pressing was maintained by the food presentation schedule, afterwhich the animals were totally frozen and stored at -70°C until analysis. The pre-tone and post-tone responding on the VII schedule were compared. The CER animal responded very little (< 1 res/min) after tone presentation, while controls continued at pre-stimulus rates (circa 15 res/min). Brains were dissected into discrete cortical and sub-cortical areas and the content and utilization of biogenic amines and amino acid neurotransmitters were evaluated. There were few changes in the content of neurotransmitters or their metabolites, suggesting that small functional pools were being utilized. Comparisons of the conditioning/emotional component (CER versus shock only) revealed a general increase in turnover of Asp, Glu, DA and NE in multiple areas and mixed changes in turnover of 5-HT and decreased turnover of GABA in limbic areas. If diazepam is reversing CER through pathways common to the paradigm, then respective compensatory reversals of turnover would be expected. This was observed in approximately one-third to one-half of the areas and transmitters investigated. Changes in turnover following diazepam enhanced the turnover related to CER in additional areas, suggesting the recruitment of some novel neural pathways. (Supported in part by Grant MH-31835).

98.7 EFFECT OF N,N-DIMETHYLTRYPTAMINE ON H-REFLEX RECOVERY CURVE. John Metz, Nash'aat Boutros*, Patricia Tueting, Charles Grimm*, and Herbert Y. Meltzer. Lab. of Biological Psychiatry, Ill. State Psychiatric Institute, and Dept. Psychiatry, University of Chicago, Chicago, Illinois.

N,N-dimethyltryptamine (DMT) is an indolamine hallucinogenic agent which has been proposed as an endogenous psychotogen. Pharmacologically, it appears to act as a serotonergic and, to a lesser extent, a dopaminergic agonist. We examined the effects of DMT on the recovery curve of the Hoffmann monosynaptic reflex (HRRC), with and without pretreatment with serotonin and dopamine antagonists. The HRRC has been shown to be abnormally high (fast) in unmedicated psychotic patients.

Subjects were 1 black and 4 white male volunteers, aged 23 to 32, with no history of psychiatric or serious physical illness. We tested the HRRC at intervals between 50 and 300 msec (Metz et al., Psychol. Med., 1980, 10:541). Stimulus presentations were controlled by a PDP 11/03 computer, which also digitized and recorded muscle responses and measured peak-to-peak amplitudes.

Subjects were administered pretreatment capsules for 3 days--either placebo (Pl) or 12 mg/day of the serotonin antagonist cyproheptadine (Cyp). The HRRC was tested on the experimental day, followed by an IM injection of 0.7 mg/kg DMT or 2 cc. saline (Sal) and a second test of the HRRC 20 minutes after the injection. Thus, subjects were tested in 4 conditions: Pl-Sal, Cyp-Sal, Pl-DMT, and Cyp-DMT. Experimental days were at least 7 days apart, the conditions were randomly presented to each subject, and the experimenters were blind concerning the pretreatment and injection schedule. Three of the subjects were also tested in a fifth condition: pretreatment was an IM injection of 0.5 mg haloperidol one hour before the DMT.

Each subject showed a clear increase in HRRC in the Pl-DMT condition (increases of 26, 30, 69, 125, and 317% above pre-DMT level, average 113%). One subject showed an increase in the Cyp-DMT condition (78% above pre-DMT). Otherwise there were only minor changes in HRRC from before to after the injection. These results were statistically significant (Friedman 2-way analysis of variance, p less than .02). All of the subjects pretreated with haloperidol showed increases in HRRC after the DMT (23, 62, and 177% increases, average 87%).

These results indicate that injected DMT produces an HRRC effect comparable to that found in unmedicated psychotic patients. The HRRC effect of DMT appears to be mediated by a serotonergic mechanism and is not blocked by a dopamine-receptor blocker.

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98.8 MHPG, CORTISOL, AND TRYPTOPHAN RATIO IN DEPRESSION.
S. R. Dunlop*, H. C. Hendrie*, P. A. Shea and H. M. Brittain* (SPON: J. I. Nurnberger). Institute of Psychiatric Research, Indiana University Medical Center, Indianapolis, IN 46223.

Subtyping of depressed patients into serotonergic or adrenergic groups using biochemical variables is of research interest. Two potentially useful variables are 24 hour urinary MHPG excretion which may reflect CNS noradrenergic activity and the plasma tryptophan ratio (the ratio of tryptophan to the other neutral amino acids which compete for entry into the CNS) which may control entry of tryptophan to the CNS and relate to serotonergic activity.

In this study, these variables and urinary cortisol excretion were measured in 33 unipolar depressed inpatient subjects during the first week of hospitalization. Subjects were also assessed for diagnosis by RDC criteria and rated on the Hamilton depression rating scale at admission and two follow-ups. Results were examined for relationships between the biochemical variables at admission and between each variable and depressive subtype, severity and change in depressive symptoms. Preliminary analyses of the results showed some significant differences between males (n=15) and females (n=18). Therefore, data were anlayzed separately by sex.

When the biochemical variables were analysed for interrelationships, in females there was a significant negative correlation between MHPG and tryptophan ratio, r=-.436, p=.01 and MHPG was correlated to cortisol in males, r=.557, p=.02.

In both male and female subjects, urinary cortisol was the only variable which related to subtype of depression. In females, cortisol excretion was higher in endogenous (29±10 μg /24 hr.) than non-endogenous (8±10) depression, p=.003, and tended to be higher in recurrent (21±14) than non-recurrent (9±6) depression, p=.06. In males, cortisol was higher in the situationally depressed (46±12) than in the non-situationally depressed (27±13), p=.05. In males and females, none of the biochemical variables correlated with Hamilton score at admission. When improvement by 50% of original Hamilton score was used to define responders, responding males had lower MHPG (1.7±.4 mg /24 hr.) than non-responders (2.5±.4), p=.058.

In summary, the biochemical markers selected in this study, one noradrenergic, one serotonergic, and one neuroendocrine marker, showed only few relationships to subtype or degree of depression in this sample of moderately depressed patients. Only MHPG at admission in males could be related to change in symptomatology. However, the inverse correlation between tryptophan ratio and MHPG could support the use of these variables in distinguishing depressed subjects.

98.9 ELEVATED MHPG RELATED TO DEXAMETHASONE RESISTANCE IN DEPRESSED PATIENTS. D.C. Jimerson, T.R. Insel*, V.I. Reus*, I.J. Kopin. Lab. of Clinical Science, and Clinical Neuropharmacology Br., NIMH, Bethesda, MD 20205; and Langley Porter Neuropsychiatric Institute, San Francisco, CA 94143.

Up to 50% of severely depressed patients show inadequate suppression of serum cortisol following dexamethasone (DEX) administration. The dexamethasone suppression test (DST) may define a state-dependent biological marker useful in diagnostic and treatment evaluation.

In this study, we have assessed the relationship between DST response and adrenergic activity in 15 drug-free depressed patients meeting DSM III criteria for major affective illness. Severity of depression and anxiety was assessed on the Symptom Checklist, Hamilton, and Brief Psychiatric Rating Scales. Plasma levels of cortisol and 3-methoxy-4-hydroxyphenyl glycol (MHPG), which is a good index of cumulative sympathetic activity, were measured simultaneously in 8 AM plasma samples. Samples were obtained on a baseline day, and on the morning following 11 PM administration of 1 mg. DEX.

A robust positive correlation was observed between post-DEX cortisol and MHPG ($r=.89$, $p<.0001$); a modest positive correlation was also observed in the baseline state. Post-DEX MHPG was higher in 6 DST non-suppressors (20.7±2.8ng/ml) than in 9 suppressors (12.4±1.0 ng/ml) ($p<.01$). There was a trend toward higher anxiety ratings in the non-suppressors; depression ratings were similar for both groups.

These data suggest that DST resistance in depressive illness is associated with increased noradrenergic activity and is not consistent with the suggestion, based on studies in animals, that a central noradrenergic deficit is responsible for the abnormal endocrine response to DEX. While elevated catecholamines may be causally related to cortisol elevation, it seems more likely that adrenergic and hypothalamic-pituitary-adrenal activation in depressed patients may reflect parallel responses to another stimulus--possibly illness-related stress.

98.10 LEARNING DISABILITY CORRELATES OF URINARY CATECHOLAMINE METABOLITES IN HYPERACTIVE BOYS. W.O. Shekim, E. Horwitz*, J. Javaid, D.B. Bylund, J.M. Davis. Mid-Mo. Mental Health Ctr., Univ. of Mo. Sch. of Med., Columbia, MO 65201.

The authors examined the relationship between urinary homovanillic acid (HVA), the main metabolite of dopamine and 3-methoxy-4-hydroxyphenylglycol (MHPG), the main metabolite of CNS norepinephrine, and several psychoeducational measures often used in the assessment of learning disabilities in 28 hyperactive boys. The children were admitted to a clinical research center and placed on a low monoamine VMA exclusion diet. Three 24-hour urine collections were made on ice in bottles containing sodium metabisulfite 0.5 gm/liter of urine. HVA was assayed according to the method of Dziedzic et al (1972) and MHPG according to the method of Dekirmenjian and Maas (1970). Each child received the following battery of tests during his admission: Wechsler Intelligence Scale for Children-Revised (WISC-R), Wide Range Achievement Tests (WRAT), Beery Developmental Test of Visual-Motor Integration (VMI), and Peabody Picture Vocabulary Test (PPVT). Test variables included traditional scores for each test as well as measures of individual scatter, discrepancy between obtained and expected performance, etc. Pearson Product-Moment Correlation Coefficients were computed between each biochemical measure and each of the test variables. A probability of $p<.05$ was required for significance on all tests. There were a number of significant correlations between HVA measures and psychological variables. On the WISC-R, higher values of HVA/m^2 were associated with higher scores on Vocabulary ($r = .49$), Digit Span ($r = .42$), and Verbal IQ ($r = .39$). There was a positive correlation between HVA/m^2 and the discrepancy between WISC-R, Verbal IQ (VIQ) minus Performance IQ (PIQ) ($r = .37$) indicating that higher values of HVA/m^2 were associated with better performance on VIQ than PIQ and lower values of HVA/m^2 were associated with better non-verbal skills than verbal skills. In addition, more scatter within the performance subtests was associated with higher values of HVA, HVA/m^2, and HVA/creatinine (r's = .37 to .40) On the WRAT, higher levels of HVA/m^2 were associated with higher standard scores on the Reading ($r = .56$) and Spelling ($r = .40$) subtests. The discrepancy between each child's school grade placement and WRAT grade level score was computed for each subtest. Significant correlations between Reading discrepancy and HVA ($r = -.70$), HVA/m^2 ($r = -.71$), and HVA/creatinine ($r = -.44$) and Arithmetic discrepancy and HVA/creatinine indicated that children with lower HVA values tended to be farther behind than those with higher HVA levels. Other significant correlations between HVA, MHPG, and test measures were also found. However, the correlations with MHPG were not as meaningful as the correlations with HVA suggesting a more direct relationship between dopamine metabolism and learning in children.

98.11 RANK-DEPENDENT CHANGES IN PRIMATE BEHAVIOR AFTER AMPHETAMINE CHALLENGE. K.A. Miczek and L.H. Gold*. Dept. of Psychology, Tufts Univ., Medford, MA 02155.

The role of brain catecholamines in processes mediating affect and integrative functions may be elucidated by studying drug action on primate social behavior. One major limitation of this approach is the marked variability in behavior among members of a social group. We investigated the influence of age, sex, and especially social rank on amphetamine-induced changes in the behavioral repertoire of squirrel monkeys. Rank was determined by constructing a sociogram on the basis of sent and received agonistic activities. Using the focal animal technique, we recorded a profile of motor activities and measured the incidence of salient social interactions for one hour after oral or intramuscular administration of d-amphetamine sulfate in 3 independent groups of squirrel monkeys (n=25). Additionally, amphetamine's action on motor elements of behavior was examined in individual monkeys placed into empty colony rooms. d-Amphetamine (0.3 - 1.0 mg/kg, p.o.) increased stereotyped head movements, and reduced the time spent in the sitting posture in all monkeys regardless of sex, age, or social rank. The high levels of locomotor activity by dominant and juvenile monkeys were decreased at higher amphetamine doses (0.6, 1.0 mg/kg, p.o.), whereas the same doses increased locomotion in otherwise less active subdominant and submissive animals. Amphetamine-induced changes in the various motor activities were closely similar in the presence or absence of group members. Subordinate and submissive monkeys showed the largest disruption of displays, displacement and grasping directed toward other monkeys, when given amphetamine (0.1 - 0.6 mg/kg, i.m.; 0.6, 1.0 mg/kg, p.o.), whereas in dominant animals these agonistic behaviors were decreased only at the highest amphetamine dose. Furthermore, we investigated the time course of 0.3 mg/kg d-amphetamine, i.m., on motor functions and a broad repertoire of social and agonistic interactions in monkeys belonging to three separate groups. Amphetamine increased the time spent distant from other group members, and decreased the total amount of social interactions in all monkeys regardless of age, sex and social rank. In subgroups of monkeys, each consisting of dominant, subdominant, submissive, and juvenile animals, amphetamine induced vocalizations akin to distress calls, and reduced elements of olfactory marking behavior such as nasal, anogenital, and back rubbing, and sneezing and urine marking. Social rank did not predict the magnitude and duration of disruption of social and agonistic behavior, at this intermediate dose. Yet, social rank appears to determine the effects of a wider range of amphetamine doses on agonistic behavior and on locomotor activation, but not on motor stereotypies. Social rank may be linked to altered catecholamine activity.

98.12 BEHAVIORAL RESPONSES TO L-DOPA IN SQUIRREL MONKEY (SAIMIRI SCIUREUS). Sherry L. Berg* (SPON: S.G. Speciale). DeVry Institute of Technology, Irving, TX 75062 and Baylor College of Dentistry, Dallas, TX 75246.

Two naive male squirrel monkeys were treated systemically with 50 mg/kg L-DOPA plus 20 mg/kg carbidopa and 4 mg/kg ascorbic acid injected i.p. Primates were confined in a 30 x 28 x 60 cm plexiglass test chamber and were viewed and filmed in the presence of the experimenter.

A comparison of the behavioral observations from the nondrug and drug conditions indicated different response repertoires. Behavior from control and saline conditions showed elements of environmental dependence, while the behavior patterns in the L-DOPA treatment appeared to be internally triggered with some aversive component, for example, scratching, maniacal grooming and face rubbing. Also, approximately one hour post-drug and for the duration of the intense activity period, behaviors that were previously recorded individually were grouped into rapidly executed stereotypic sequences. (Supported by Huntington's Chorea Foundation and NINCDS Grant NS 15020)

99.1 FMRFAMIDE INCREASES CNS CONTROLLED GILL WITHDRAWAL BEHAVIORS AND ASSOCIATED NEURAL ACTIVITY IN APLYSIA. K. Voshart* and Ken Lukowiak*, (SPON: T. P. Hicks). Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

In Aplysia, the gill withdrawal reflex (GWR) can be evoked by a tactile stimulation to the siphon and it habituates. It is possible in a semi-isolated preparation, to study central nervous system modulation and control of gill movements and habituation. Based on the GWR it is possible to operationally define the "behavioral state" of the preparation. The three behavioral states that have been described are the facilitated, normal and suppressed states. These classifications are based on the comparison of the gill reflex amplitude (stimulus intensity = 1 g) to amplitude of the centrally mediated spontaneous respiratory gill movement (SGM). The suppressed state is characterized by small GWR, < 35% of SGM, and rapid rate of habituation. The facilitated state is characterized by GWR > SGM and the reflex is resistant to habituation. A preparation in the normal state habituates and the GWR amplitude is > 35% but < 100% of an SGM. Previous reports from this laboratory have described CNS mediated suppressive effects of neuropeptides on these gill reflex behaviors. Arg-vasotocin, Arg-vasopressin and Met-enkephalin suprafused over the abdominal ganglia reduced the amplitude of the tactile-evoked responses and increased the rate of reflex habituation. (J. Neurobiology 12, 1981, Reg. Peptides 3, 1982). We now report that molluscan cardio-excitatory neuropeptide, FMRFamide (10^{-9}M - 10^{-6}M) suprafused over the CNS potentiated the tactile-evoked GWR, disrupted habituation and induced gill movements.

The preparation consisted of the siphon, mantle, gill and abdominal ganglion. The abdominal ganglion was connected via the siphon, ctenidial and branchial nerves. A chamber surrounding the CNS allowed selective CNS application of peptides. Threshold concentrations of FMRFamide required to produce the different behaviors varied. The amplitude increase of the tactile evoked response was the most sensitive measure of FMRFamide effects. Fifty percent increases were seen at 10^{-9}M. Four hundred percent increases were seen at 10^{-6}M. The rate of spontaneous firing of gill motor neurons L_7, LDG_1, and L_9 usually increased. Habituation could be blocked at 10^{-6}M and habituation rate was reduced at 10^{-7}M. Intense contractions of the gill comparable in amplitude to SGMs were frequently seen in response to 10^{-7}M peptide application. It therefore appears that FMRFamide, applied to the CNS, changes the behavioral state of normal preparation to the facilitated state.

This work was supported by the MRC of Canada and the AHFMR.

99.2 AVERSIVE PROPERTIES OF VASOPRESSIN MAY ACCOUNT FOR ITS PUTATIVE ROLE IN MEMORY. A. Ettenberg, D. Van der Kooy, M. Le Moal, G.F. Koob and F.E. Bloom. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute, San Diego, California 92138.

Many recent reports have suggested that experimentally-applied arginine vasopressin (AVP) improves test performance by acting directly on the neural mechanisms involved in the processing and storage of new information. However, without knowledge of the precise molecular and cellular events involved in "memory" such interpretations of AVP action may overlook testable "non-memory" explanations. In the present study we examined the possibility that aversive consequences of AVP administration might account for its apparent memory-enhancing properties.

The aversive properties of AVP were demonstrated using three behavioral assays: 1) AVP (0.0, 1.2, 6.0, or 20.0 ug/kg s.c.) produced a dose-dependent decrease in spontaneous locomotor activity. 2) Presentation of a novel tasting solution (saccharin) to thirsty rats followed by an injection of either vehicle or AVP subsequently resulted in an AVP-induced conditioned taste aversion in which rats avoided a normally preferred substance when its presentation was previously paired with AVP administration. 3) In a conditioned place test, rats learned to avoid a distinctive environment previously associated with AVP administration. Taken together, these data suggest that doses of AVP reported to produce behavioral effects consistent with a memory-enhancing action concurrently produce aversive consequences in treated animals.

Last year (Koob et al. Neurosci. Abstr., Vol. 7, p.30, 1981) we reported that rats injected with AVP immediately after exposure to a novel open-field environment found a water tube in that same environment faster than controls when water-deprived 48 hrs later. In the present study, additional groups of rats were tested with comparable doses of the AVP- analog des-glycinamide arginine vasopressin (DGAVP) in the appetitive water-finding task and in the three behavioral tests described above. DGAVP, said to have little or no peripheral endocrinological actions, produced no evidence of any aversive properties in the present study. However, DGAVP also did not alter the performance of rats in the appetitive memory task. Based on these data we hypothesize that: a) the peripheral physiological actions of AVP (e.g. pressor response) are responsible for its aversive properties, and b) such aversive consequences may be necessary to demonstrate improved test performance. Thus the memory-enhancing properties of AVP might reflect the arousing or alerting effects of administering an aversive agent. This research was supported by federal grants NIDA 01785 and NIAAA 03504.

99.3 ALPHA-MELANOCYTE STIMULATING HORMONE FACILITATES LEARNING OF VISUAL BUT NOT AUDITORY DISCRIMINATIONS. Gail E. Handelmann*, Thomas L. O'Donohue, David Forrester*, and Walter Cook* (SPON: David Symmes). Lab. of Clinical Science, NIMH, Bethesda, MD, and Dept. of Psychology, The Johns Hopkins Univ, Balto., MD.

Alpha-melanocyte stimulating hormone (MSH) is a neuropeptide known to facilitate learning of a variety of tasks. Evidence from animal and human studies suggested that MSH might influence learning by selectively improving visual attention. To directly test this hypothesis, rats injected with either MSH (40 µg/kg) (n=24) or saline (control, n=24) were tested in one of four discrimination tasks. Each task was a two-choice discrimination using food rewards as reinforcers. Two of the discriminations were conducted in a Y-maze. One used visual cues (black and white curtains) to signal the location of the reward, and one used auditory cues (two tones of different frequencies). The other two tests were conducted in an operant chamber with bars to press to obtain the reward. One task used visual cues (a steady or blinking light) to signal which bar to press, while the other used auditory cues (two tones of different frequencies). Each rat was trained for acquisition and reversal of one discrimination until it reached a criterion of choice accuracy.

In both visual discriminations, the rats injected with MSH learned the tasks faster than did the controls. The groups did not differ in their rates of reversal. In the auditory discriminations, the rats injected with MSH learned the tasks at the same rate as the controls, and the groups did not differ in their rates of reversal.

Effect of MSH on Rate of Discrimination Learning
Days to Criterion

	Visual Cues		Auditory Cues	
	Control	MSH	Control	MSH
Y-Maze	6 ± 0	3 ± 0*	17 ± 5	15 ± 4
Bar-Press	31 ± 2	17 ± 2*	11 ± 2	14 ± 2

*Different from Control, Mann-Whitney U, p<.05

Regardless of the type of response required of the rat in the task, MSH injections facilitated learning of visual discriminations but not auditory discriminations. MSH therefore appears to influence learning by selective facilitation of visual attention and/or processing of visual information.

99.4 MEMORY ENHANCEMENT INDUCED IN CHICKS BY L-PROLYL-L-LEUCYL-GLYCINE AMIDE AND OXYTOCIN. J.L. Davis, R.M. Pico* and A. Cherkin. Aging and Behav. Biol. Res. Lab., V.A. Med. Ctr., Sepulveda, CA 91343.

We have previously described certain proline oligopeptides to be enhancing in a conditioned peck aversion paradigm using methyl anthranilate (MeA) as the aversant (Davis, J.L. and Cherkin, A., Neurosci. Abstr., 6: 169, 1980). We report here an expansion of these results to include a demonstration of the retrograde nature of the PLG enhancement in a similar paradigm. Furthermore, we also describe retrograde enhancement of memory with the neuropeptide oxytocin, of which PLG represents the C-terminal tripeptide.

In these experiments two-day-old cockerels were injected intracerebrally with 5 µl/hemisphere of PLG (3.0 µmol/chick), oxytocin (0.01 unit/chick, 20 ng) or saline 1 min after one-trial training to suppress the peck response to a bead coated with EtOH whose less potent aversant effects are not noticeable 24 hr later without a memory enhancement manipulation. Enhanced memory was noted, 24 hr later, when chicks showed a lower mean peck rate (number of pecks in 10 sec) than the control chicks when presented with an uncoated bead. Further data (not shown here) indicate that decreased reponding is not the result of a general depressive effect of the peptides.

Post-Training Injection(min)	Compound	N	Retention Test $\bar{P}$ ± SD		p values
1	PLG	50	5.58	7.98	<0.01*, 0.05†
9	PLG	49	7.35	6.74	NS*
59	PLG	49	9.71	7.37	NS*
1	Saline	49	8.84	6.54	

*Significance levels obtained by Dunnett comparisons with Saline group.
†Significance levels obtained by Tukey-HSD comparison of the 1 min PLG group to the 59 min PLG group.

Post-Training Injection(min)	Compound	N	Retention Test $\bar{P}$ ± SD		p values
1	Oxytocin	59	7.19	7.01	<0.05*†
9	Oxytocin	58	8.22	6.61	NS*
59	Oxytocin	59	10.00	6.66	NS*
1	Saline	60	10.25	7.01	

*Significance levels obtained by Dunnett comparisons with Saline group.
†Significance levels obtained by Tukey-HSD comparison of the 1 min Oxytocin group to the 59 min Oxytocin group.

These results indicate that memory can be improved in this avian model with either oxytocin or its C-terminal peptide PLG.

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99.5 VASOPRESSIN DEFICIENCY, OPERANT BEHAVIOR, AND QUININE-INDUCED DRINKING SUPPRESSION. G.N.O. Brito, M.E. Stanton*, G.J. Thomas and D.M. Gash. Center for Brain Research, University of Rochester School of Medicine & Dentistry, Rochester, NY 14642.

Performance of vasopressin-deficient rats (Brattleboro strain--DI) and normal (LE) rats were compared on water-motivated operant behavior and quinine-induced drinking suppression. One group of DI (N=5) and one group of LE (N=5) rats were adapted to the laboratory for 2 weeks. During the first week, measures of 24-h water intake clearly established the diabetes insipidus status of the DI rats. For the remainder of the study, DI rats were water deprived for 5 1/2 h and LE rats were deprived for 23 1/2 h. This water-deprivation regimen induced a significant increase in urine osmolality of both groups with DI rats showing much lower osmolalities for both hydrated and dehydrated conditions. The water-deprived rats were then placed once a day (for 1 to 10 min) for 11 days in a standard operant chamber containing a dish of water. Following adaptation to the operant chamber and shaping, the rats were run under the following fixed-ratio (FR) schedules: FR-1 (continuous reinforcement), FR-2, FR-4, FR-5, FR-7, FR-8 and FR-10. To advance to higher FR, the rat was required to make at least 100 responses on 3 consecutive sessions of 15 min duration each. After the rats performed the FR-10 schedule, they began a progressive-ratio (PR) schedule on which the rats had, on a given session, to press the lever 6 times to obtain the first reinforcement, 12 times for the second, 24 times for the third, 36 times for the fourth, etc. Following the PR schedule, the rats were tested for quinine-induced drinking suppression. This test consisted of measuring intake in the home cage for 15 min on successive days of water and quinine (0.0005; 0.001; 0.01; 0.1 g%).

The results showed that 6-h deprived DI rats responded significantly less than 24-h deprived LE rats on FR-5, FR-7 and FR-10, and their last completed FR on the PR schedule was significantly lower than that of LE rats. However, there was no significant difference between DI and LE rats in magnitude of quinine-induced drinking suppression.

We conclude that 6-h deprived DI rats are less likely to press a lever for water than 24-h deprived LE rats, even though they are more severely in negative water balance than LE rats. However, DI rats are not "finicky" at least as determined by quinine-induced drinking suppression. It is likely that timidity interferes with the behavior of DI rats in the operant chamber.

Supported by NIH grant NS-17543.

99.6 MET^5-ENKEPHALIN REDUCES NEOPHOBIA IN FETAL RATS. G.Stickrod* (SPON: D.P. Kimble) Dept. of Psychol., Univ. of Oregon, Eugene, OR, 97403 and W.P. Smotherman Dept. of Psychol., Oregon State University, Corvallis, OR, 97331.

Rat pups will avoid taste/odor stimuli they experience in-utero if the in-utero exposure is paired with LiCl toxicity. Taste/odor aversions can be conditioned in-utero (Stickrod, Kimble and Smotherman, Physiology and Behavior, 1982). In this experiment female Sprague-Dawley rats were anesthetized at Day 20 of gestation and uterine horns exposed by midline laparotomy. Fetuses were exposed to an apple juice or saline solution (.04 ml) by amniotic injection. Following the last amniotic injection fetuses were injected i.p. with LiCl (.02 ml/.15M) or the volumetric equivalent of saline. After these i.p. injections fetuses received a second i.p. injection of Met^5-enkephalin in a dosage of 80 µg/kg in .02 ml or the same volume of saline. The 4 Treatment Groups resulting from the combination of 2 amniotic injections (apple solution/SAL) with 2 i.p. injections (LiCl/SAL) were subdivided for the second i.p. injection (Met^5-enkephalin/SAL). This yielded a total of 8 Treatment Groups (N=3 litters/Treatment Group). Litters were taken by Cesarean section on Day 22 of gestation and fostered to females that had delivered a litter within 24 h. On postnatal Day 16/17 pups were tested individually in a wire mesh enclosure that was placed over a two-chambered box that held unscented alder shavings (in one chamber) or shavings scented with the odor of apple solution. Pups were placed in the center of the enclosure and allowed free movement for 100 sec. Time spent over the odor emanating from the apple solution was calculated. These data were compared in a 4 (Treatments: SS,AS,SL, AL) by 2 (Conditions: Met^5-enkenphalin, SAL) ANOVA. The Treatments by Conditions interaction was significant $F(3,16)=8.85$, $p<.05$. The post-hoc comparison for Treatment effects within the SAL condition indicated that pups in the AL treatment showed a decreased preference for the side of the chamber over the apple odor compared to SS, SL, and AS controls (p's$<.05$) that did not differ from one another. Within the Met^5-enkephalin treatment pups receiving the AL treatment avoided the side of the chamber over the apple odor compared to SS, AL (p's$<.05$) and AS ($p<.01$) control groups. The AS group showed an increased preference for the apple odor. Data indicated that pups form an odor aversion when an in-utero taste/odor exposure is followed by LiCl injection Met^5-enkephalin did not signivicantly affect the magnitude of the aversion. However, animals exposed to the apple odor in-utero and injected with Met^5-enkephalin did show an increased preference for the apple odor postnatally. Data are supportive of a memory or attentional hypothesis of peptide action. (Supported by NICH and HD grant HD 16102-01 to WPS).

99.7 INFUSION OF B-ENDORPHIN INTO THE VENTRAL TEGMENTUM OF RATS INCREASES RATE OF AVOIDANCE RESPONDING. C. Ksir and P. Sanderson*. Department of Psychology, University of Wyoming, Laramie, WY 82071.

Infusion of small amounts of B-endorphin (BE) into the ventral tegmental area (VTA) of rats has been shown to increase locomotor activity in photocell cages (Stinus, Koob, Ling, Bloom and LeMoal, Proc. Natl. Acad. Sci. 1980). These injections are made in the region of the A10 group of dopamine cell bodies, and demonstrate an interaction between opiate and dopamine systems at that location. Similar injections were used (Schwartz, Ksir, Koob and Bloom, Neurosci. Abstr. 1981) to examine changes in responsiveness to opiate stimulation following pre-treatment with large doses of morphine. The use of activity as a measure of these effects is convenient, but is complicated in that any drug effect must be measured against a changing baseline of activity over time. The current experiments employed rats trained to postpone electric shocks by pressing a lever. This continuous avoidance schedule provides a stable baseline against which to evaluate the stimulant or depressant actions of a drug (Heise and Boff, Psychopharmacol. 1962).

Fourteen adult male hooded rats were trained to respond on a schedule in which shocks were delivered every 5 sec and each lever press postponed shock for 20 sec. Each rat was trained in 4-hour sessions once a week until it avoided 90% of the shocks in a session. The rats were then anesthetized and implanted bilaterally with stainless steel 26 ga cannulae aimed at the VTA. After recovery, each rat was reintroduced to the avoidance schedule for 1 hour, bilateral infusions of either 0.5 ug or 1.0 µg BE were made into the VTA, and the rat was then tested for the next 3 hours. Each rat was tested weekly until data were obtained for each animal with 0.5 and 1.0 µg infusions of BE, vehicle infusions, and 1.0 µg BE after a systemic injection of naloxone (1 mg/kg, s.c.). Infusions of either 0.5 or 1.0 µg BE per side (total of 0.3 or 0.6 nM BE per rat) produced increases in lever-pressing lasting for at least 90 min. Naloxone blocked the effect of 1 µg BE infusions.

99.8 TOLERANCE DEVELOPS TO THE ANTIAVOIDANCE PROPERTIES OF CHOLECYSTIN OCTAPEPTIDE. S.L. Cohen*, C.A. Tamminga, M. Knight and T.N. Chase.

Behavioral analysis of the activity of cholecystokinin octapeptide (CCK8) has suggested that CCK8 can alter classic behaviors in the laboratory rat in a manner similar to that produced by haloperidol, a potent blocker of DA receptors. Since the discovery that CCK8 may coexist with DA in certain of the mesolimbic tract DA neurons, we have been exploring possible actions of CCK8 in brain and how they may relate to dopamine system function. We have previously reported a neuroleptic-like effect of CCK8 on conditioned avoidance learning in rats; dose sensitivity of this response using a Sidman avoidance technique is in the range of 40-640 µg/kg with intraperitoneal injection. This behavioral action of CCK8 fails to obtain with either the nonsulfated form of CCK8 or with gastrin-4, a peptide with some peripheral actions similar to CCK8. Studies were undertaken to determine whether or not tolerance develops to this CCK8-induced action on Sidman conditioned avoidance. Sixteen female Sprague-Dawley rats were trained to avoid electric shock in a free-operant (Sidman) avoidance paradigm for 8 sessions. Then CCK8 (320 µg/kg) or saline was administered intraperitoneally twice daily for 7 days to the trained animals. When this chronic treatment sequence was completed, each group was tested in the Sidman avoidance paradigm following an acute dose of CCK8 (640 µg/kg) or saline. Four drug-treatment groups were developed: saline-saline, saline-CCK8, CCK8-saline, and CCK8-CCK-8. Analysis of the trained avoidance performance of each animal group prior to the 7-day drug treatment indicated that no group differences existed prior to dosing. However, when tested after chronic CCK8 or saline, the anti-avoidance effect of CCK8 was manifest only in those rats treated for seven days with saline. No anti-avoidance effect of CCK8 was apparent in those animals treated with subchronic CCK8. A split-plot analysis of variance performed on the data showed a significant acute drug effect of CCK8 ($p < .05$) and a significant group-test drug interaction ($p < .05$); the later analysis supports the contention that CCK8 depresses responses only if rats had no prior experience with CCK8. We conclude that, with respect to this behavioral measure, tolerance develops to the actions of CCK8 after 7 days of continuous treatment, in a manner similar to that reported for the appetite suppressant action of CCK8.

99.9 HIPPOCAMPAL VASOPRESSINERGIC TERMINALS ARE MODULATED BY PERIPHERAL VASOPRESSIN THROUGH SHORT-TERM NEGATIVE AND LONG-TERM POSITIVE FEED-BACK MECHANISMS. Adolfo G. Sadile. Inst. Human Physiol., Univ. Naples, 1st Med. Sch., Naples, Italy.

The widespread vasopressinergic fibers to the entire CNS constitute an heterogeneous system for vasopressin-mediated central functions, yet to be ascertained (Buijs, R.M., Cell Tissue Res., 192: 423, 1978). Moreover, since about nothing is known as to its control mechanisms and there is scarce evidence for vasopressin (VP) hormone crossing the blood-brain barrier, in spite of redundant literature on peripherally-administered VP affecting behaviour, we focused on plasma VP level as one peripheral signal in controlling the central system. For this purpose we carried out a series of electrophysiological and behavioural studies in the adult albino rat after having changed the plasma VP level either endogenously a) by water-deprivation periods of different length (WD: 12,24 or 48hr; b) or taking advantage of the seasonal fluctuations (high VP during summer-fall, low during winter-spring) or exogenously by parenteral supply, as a single, acute or multiple, sub-chronic injections (twice daily for 7 days) with dose-response analysis.
Electrophysiologically, in acute pentothal-anaesthetized preparations, microinjecting threshold doses of VP in CA4-area dentata region and recording extracellularly the elicited slow field potential, we found the sensitivity to VP (in the range of 10^{-9} to 10^{-6}M) to be negatively correlated in the short-term and positively in the long-term with the plasma VP level.
Behaviourally, retention of habituation to a novel environment (LT-HAB) was facilitated by high VP plasma level in the post-trial period either with WD-48hr or during summer-fall or with high doses of exogenous VP and interfered with by low level increase either with WD-12hr or during winter-spring or low doses of VP.
The experimental evidence fits well within the hypothesis of central VPergic systems being controlled by plasma VP through phasic, negative and tonic, positive feed-backs adjusting the sensitivity of vasopressin receptor(s) to centrally released VP. Thus, plasma VP would be one peripheral signal modulating the central VPergic system, which would in turn have a modulatory role in the control of signal traffic across neural membranes through a wide variety of excitability changes to external and internal inputs, at various organizational levels in the CNS.

100.1 VASOPRESSIN PRESSOR ANTAGONIST REVERSES CENTRAL BEHAVIORAL EFFECTS OF VASOPRESSIN. M. Le Moal, G.F. Koob, P. Mormède*, R. Dantzer* and F.E. Bloom. Lab. Neurobiologie des Comportements, Université de Bordeaux II, Bordeaux, France, and A.V. Davis Ctr for Behavioral Neurobiology Salk Inst. San Diego, California 92138.

Previous work (Le Moal et al., Nature, 291, 491, 1981) from our studies has established that the prolongation of extinction of active avoidance produced by arginine vasopressin (AVP) injected subcutaneously (s.c.) could be reversed by pretreatment with an analogue of vasopressin, 1-deaminopenicillamine-2- (O-methyl)-tyrosine arginine vasopressin (dPTyr (Me) AVP) that acts as an AVP pressor antagonist. Our previous work also showed that much smaller doses of AVP (1 nanogram/rat) injected intracerebroventricularly (i.c.v.) also prolonged the extinction of active avoidance (Koob et al. Reg. Peptides 2, 153, 1981). The purpose of the present study was to examine the effects of this vasopressin antagonist, injected peripherally, on the prolongation of extinction produced by central, i.c.v., injection of AVP. Male, Wistar rats were equipped with a chronic cannulas aimed above the lateral ventricle. One week following surgery, the rats were trained to jump on a pole to avoid shock in a series of 10 trials on each of 3 successive days. On the fourth day a series of extinction tests were conducted every two hours. Ten trials in succession were given to each rat with an intertrial interval of 40, 60, or 80 secs. Following the first 10 trials of extinction, each rat was placed into one of 3 groups and received either a peripheral injection, s.c., of saline and a central injection (2 ul) of saline (SAL/SAL Group), a peripheral injection of saline, s.c., and a central injection of 1 nanogram of AVP (SAL/AVP group), or a peripheral injection of dPTyr (Me) AVP, s.c., (30 micrograms/kilogram) and a central injection of 1 nanogram of AVP (dPTyr (Me) AVP/AVP group). Results showed that dPTyr (Me) AVP completely reversed the effects produced by central injection of AVP. The SAL/AVP rats continued to jump an average of 6-7 avoidances up to 8 hours post-injection, whereas the SAL/SAL and dPTyr (Me) AVP/AVP rats had virtually extinguished by 4 hours post-injection. These results suggest that central injection of AVP produces visceral arousal signals similar to that produced by peripheral injections of AVP and that these signals are somehow related to the action of AVP in increasing blood pressure.

The results also suggest an intimate relationship between the central and peripheral actions of AVP in order to produce an integrated behavioral response, particularly in response to aversive situations.

100.2 STRESS INDUCED ACTIVATION: BIOCHEMICAL AND BEHAVIORAL MEASURES. Z. H. Galina*, C. Sutherland*, and Z. Amit. Dept. of Psychology. Concordia University, Montreal, Quebec, Canada.

Three experiments were performed in order to analyse the behavioral and biochemical correlates of four different intesities of the same stressor. In experiment 1, rats were exposed to heat stress (hot-plate) of varying temperatures (21,47,52,57°C) for 30 seconds. Activity was recorded in an open field immediately after stress for 30 min and revealed that the milder temperatures increased, while the higher temperature decreased activity. Experiment 2 accessed the pituitary-adrenal response to the different temperatures by measuring levels of plasma corticosterone. The four levels of hot-plate temperatures induced differential levels of corticosterone which may best be described as a U-shape function, with the extreme temperatures inducing the highest levels of the steroid. Experiment 3 further manipulated to pituitary-adrenal axis by administering dexamethasone (0.4 mg/kg 25 hrs and 0.2 1 hr before) and ACTH (20 iu, 1 hr before. Both affected activity levels following the milder stress but not the severe stress. This indicates that the decreased activity levels after severe stress may not be mediated by pituitary hormones known to be blocked by dexamethasone, yet milder stress procedures are affected by these same manipulations.

100.3 SOURCES OF VARIANCE IN OXYTOCIN-INDUCED MATERNAL BEHAVIOR. J. A. Ascher*, C. A. Pedersen*, D. E. Hernandez and A. J. Prange, Jr. (SPON: R. A. King). Department of Psychiatry and Biological Sciences Research Center, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27514.

Source of rats: In virgin Sprague-Dawley female rats from Zivic Miller Laboratories (ZMSDs) we found that ICV injection of 400 ng of oxytocin (OXY) 48 hrs after ovariectomy (OVX) and sc injection of 100 µg/kg of estradiol benzoate (EB) produced a 72% (77/107) incidence of full maternal behavior compared to 18% (9/51) after normal saline (NS). This method was ineffective in inducing full maternal behavior in Sprague Dawleys from Charles River breeders (CRSDs).

Estrogen treatment: A longer period of administration produced sensitivity to the maternal behavior effects of OXY in both ZMSD and CRSD rats. Virgin female rats (250-300 gm) were injected sc with 3 µg EB in corn oil at 8 a.m. each day of a ten day period starting one week after OVX. Seventy-two and 48 hours prior to ICV injection at 1 p.m. on the 10th day of EB treatment, each animal received sc 24 µg EB in corn oil. Rats from ZM received ICV either 400 ng of OXY in 10 µl of NS or NS alone. Rats from CR received ICV either 800 ng of OXY in 10 µl of NS or NS alone (800 ng is a toxic dose in ZMSDs but not in CRSDs). In the two hr observation period after ICV injection and initiation of pup contact 57% (17/30) of ZMSDs receiving OXY but only 27% (8/30) receiving NS became fully maternal ($p < .02$). Fifty percent (10/20) of CRSDs receiving OXY and only 17% (3/18) receiving NS became fully maternal ($p < .04$).

Estrous state at time of OVX: Comparison of vaginal smears obtained at the time of OVX with behavioral responses after ICV injections suggest that in CRSDs estrous state at OVX influences subsequent sensitivity to OXY. Charles River SDs in metestrus or diestrus (M-D) at OVX responded at a rate of only 14% (1/7) after OXY compared to 13% (1/8) after NS. Charles River SDs in proestrus or estrus (P-E) at OVX responded at a rate of 69% (9/13) after OXY but only 20% (2/10) after NS ($p < .03$). Thus, OXY was more effective in CRSDs in P-E than in those in M-D at OVX ($p < .03$).

Purity of OXY: We have compared the purity of three lots of OXY (as determined by HPLC) with their efficacies in inducing maternal behavior after ICV injection of 400 ng into ZMSDs OVXed and EB-primed 48 hr prior to OXY administration. Lot R2622 from Bachem Inc. induced only a 45% (13/29) incidence of full maternal behavior while lot #9721 from Bachem and a lot synthesized by Dr. Victor Hruby induced 70% (26/37) and 73% (51/70) respectively ($p < .04$, $p < .01$). Lot R2622 was highly contaminated while the other two lots contained pure OXY. (Work on this project was supported by NIMH grants MH33127, MH22536 and NICHHD grant HD16159.)

100.4 CENTRAL ADMINISTRATION OF CORTICOTROPHIN-RELEASING FACTOR ANTAGONIZES THE ETHANOL-INDUCED IMPAIRMENT OF THE RIGHTING REFLEX IN THE RAT. J.R. Wenger, E.C. Alwerud*, J. Rivier*, W. Vale and G.F. Koob. Alcohol Research Center, Salk Institute, San Diego, CA 92138.

Injection of ethanol is known to stress rats, as defined by the release of ACTH from the pituitary. The functional significance of this is unknown. Recent work from this laboratory has demonstrated that corticotrophin-releasing factor (CRF) has behaviorally and electroencephalographically activating properties suggesting that CRF might also have antisedative effects. This hypothesis was tested in the present study by injecting CRF intracranially (i.c. or i.c.v.) into male Wistar rats just prior to an i.p. injection of ethanol 3 g/kg and then periodically testing their righting reflexes.

In the first experiment, one experimenter injected the rats i.c. with CRF (0.3, 1.0, or 3.0 nmols, i.e., 2, 6.67 or 20 ug) in saline (25 ul) or saline alone ($n \geq 6$) per group. A second experimenter, who was experimentally "blind" as to the CRF treatments, then injected the rats with ethanol 3 g/kg i.p. and tested their righting reflexes. The rats treated with CRF 1 nmol were significantly less impaired than the controls at 1, 2 and 3 hr ($p<.05$). The rats treated with CRF 3 nmol were significantly less impaired at 2 and 3 hr. The antagonism appeared to be dose dependent at both 2 and 3 hrs for both the 1 and 3 nmol doses. Thus, i.c. administered CRF can antagonize ethanol-induced sedation as assayed by the righting-reflex measure.

A second experiment using hypophysectomized rats with lateral ventricular cannulae showed similar effects at doses of 0.15 and 1.5 nmoles (1 and 10 ug) at 90 and 120 min.

A role for pituitary ACTH or β-endorphin secretion in mediating this effect is excluded, since these rats lacked hypophyses. This suggests that a neural mechanism mediates this effect. Experiments are currently underway to determine whether this effect is centrally or peripherally mediated. Supported by AA07273, AA03504 and AM 26741.

100.5 OPPOSITE BEHAVIORAL EFFECTS OF N- AND C-TERMINAL FRAGMENTS OF SUBSTANCE P. M. E. Hall* and J. M. Stewart. Dept. of Biochemistry, Univ. of Colorado Sch. of Med., Denver, CO 80262.

Substance P (SP) has been reported to produce a variety of behavioral effects. We report here that in addition to the parent undecapeptide, the N-terminal fragment SP(1-7): (Arg-Pro-Lys-Pro-Gln-Gln-Phe) and the C-terminal fragment analog <E-SP(7-11): (pyroGlu-Phe-Phe-Gly-Leu-Met-amide) show effects on behavior when administered i.p. in low (0.6nmol/g) doses. These effects may mimic or be opposite to the effects seen with SP. The duration of fighting seen in male mice after prolonged isolation is significantly reduced by both SP and SP(1-7), while fighting is increased by <E-SP(7-11). Simultaneous administration of Naloxone blocks the effect of <E-SP(7-11), enhances the effect of SP and does not alter the effect of SP(1-7). Grooming in male mice is increased by SP and <E-SP(7-11), while SP(1-7) significantly decreases grooming. SP(1-7), like SP, induces a mild, Naloxone-reversible antinociception, as measured by latency to hind-paw-lick on the hot plate. The C-terminal peptide appears to be generally excitatory, while the N-terminal fragment appears to inhibit several behavioral activities. These peptides, SP(1-7) and <E-SP(7-11), or similar ones, may be produced from SP by action of known brain enzymes. Our data suggest that some central SP receptors may recognize a different part of the molecule than do peripheral SP receptors (which are thought to recognize only the C-terminal part of the SP molecule), that metabolism to active fragments may be important in SP action, and that SP activities in vivo may be quite complex. (Supported by grant NS-09199 from the NIH)

100.6 COMBINED MONOSODIUM GLUTAMATE AND BIPIPERIDYL MUSTARD LESIONS PREVENT NALOXONE BLOCKADE OF STRESS-INDUCED HYPERTHERMIA AND PRODUCE OBESITY IN RATS. A.C. Scallet and J.W. Olney, Dept. of Psychiatry Washington University School of Medicine, St. Louis MO 63110.

Neonatal monosodium glutamate (MSG) deletes a portion of the pro-opiomelanocortin (POMC) producing cell bodies of the arcuate hypothalamus (AH), but spares some immunostainable POMC neurons extending laterally into the ventromedial hypothalamus (VMH). We are exploring a more complete chemical lesioning approach by combining MSG with bipiperidyl mustard (BPM), long known to produce VMH lesions in mice and recently reported effective in rats as well (Lawton & Powley, Brain Res 221, 415, 1981). Results are monitored by behavioral screening and immunohistochemical evaluation of the lesions.

In normal rats, an acute rise in body temperature occurs in response to the "stress" (rectal probing and brief restraint) associated with taking a rectal temperature. This response referred to here as stress-induced hyperthermia (SIH), is blocked by pretreatment with naloxone (10 mg/kg sc)(Goldstein & Lowery, Life Sci 17, 927, 1975). We have found that the SIH response is readily elicited in either our controls or MSG or MSG/BPM lesioned rats but naloxone blocks this response only in controls. The failure of naloxone to block SIH in lesioned rats does not imply a general insensitivity to naloxone since lesioned rats showed a normal naloxone reversible hyperthermic response to morphine (5 mg/kg sc). It appears, therefore, that the mediobasal hypothalamic neurons deleted by MSG or MSG/BPM play a critical role in mediating naloxone inhibition of a stress-related temperature response but play no role in a separate regulatory pathway through which morphine exerts a naloxone-reversible temperature elevation.

It seems likely that the specific neurons that mediate naloxone blockade of SIH lie more in the AH than VMH portion of the mediobasal hypothalamus since BPM lesioned animals which have primarily a VMH lesion with minor overlap into AH had only a partial loss, whereas MSG/BPM animals had a complete loss of naloxone blockade of SIH. The extent to which naloxone blockade of SIH is lost in any particular animal may serve as a simple behavioral test of the extent to which POMC neurons located in the AH and bordering VMH areas are destroyed by our chemical lesioning approach. Since MSG/BPM rats developed obesity on an accelerated schedule compared to either MSG or BPM rats, the degree of obesity (as measured by Lee Index) may also be a convenient index of the extent to which the lesion has eliminated a full complement of AH plus bordering VMH neurons. Definitive clarification of the extent to which obesity or loss of naloxone SIH blockade correlates with POMC cell loss awaits completion of POMC immunohistochemical studies. Supported by USPHS grants DA-00259, ES-07066 and RSA MH-38894(JWO).

100.7 EFFECTS OF CALCITONIN ON THE CENTRAL NERVOUS SYSTEM. M.J. Twery*, C.W. Cooper*, and R.B. Mailman (SPON: M.A. Lipton). Depts. of Pharmacology & Psychiatry and Biological Sciences Research Center, University of North Carolina, Chapel Hill, NC 27514.

The putative physiological role of calcitonin (CT) is to decrease blood calcium through an action on bone. However, specific CT binding sites exist in other tissues, including brain. Changes in prolactin release, analgesia, and decreases in food consumption have been reported to follow the direct administration of CT into the brain. The present studies demonstrate that, in addition to suppressing food consumption, CT also depresses running wheel and amphetamine-induced activity. METHODS: Synthetic salmon CT (sCT, 1.4U, 100pmoles) or vehicle was administered by intracerebroventricular injection. Food consumption was measured for each 24hr period. The activity of adult male rats housed in cages with adjoining vertical running wheels was recorded as rotations per 24 hours. Doughnut-shaped cages equipped with photocell detectors were used to estimate the locomotor activity induced by IP administration of 1mg/kg amphetamine or apomorphine. RESULTS: 1) CT depressed running wheel activity and food consumption after each of four consecutive daily administrations. 2) Amphetamine treatment increased the average number of activity counts for a sixty minute period 2.5 fold. CT significantly decreased the total number of counts produced by amphetamine more than 65% ($p<0.05$). 3) Apomorphine also produced a 2.5 fold average increase in the total number of activity counts. However, the apomorphine-stimulated activity was not blocked by simultaneous administration of CT. DISCUSSION: The results show analgesia and suppression of feeding are not the only behavioral effects of CT on the CNS. Since amphetamine but not apomorphine-induced activity was depressed, CT's mode of action may alter the function of presynaptic monoamine neurons. The results indicate that sCT may be among the most potent anti-amphetamine agents of which we are aware. These findings, in concert with reports of immunoreactive CT in the pituitary, specific binding sites for CT in brain, and other behavioral effects of CT, increase the likelihood that a CT-like molecule may act as a neurotransmitter-modulator in CNS.

100.8 INTERACTIONS OF NEUROTENSIN WITH DIRECT AND INDIRECT DOPAMINE AGONISTS: NEUROCHEMICAL AND BEHAVIORAL MEASURES. D. Luttinger, D. E. Hernandez, R. B. Mailman, C. B. Nemeroff, and A. J. Prange, Jr. Biological Sciences Research Center, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

Considerable evidence supports the hypothesis that centrally administered neurotensin (NT) interacts with brain dopaminergic systems. Evidence for NT-dopamine (DA) interactions is the increased DA turnover observed after intracisternally (IC) administered NT. In addition, methylphenidate-induced increase in locomotor activity, which is believed to be due to increased DA release, is attenuated by IC NT in rats and mice. Administration of a direct DA agonist, apomorphine, also increases motor activity. However, this effect is not altered by IC NT.

The present studies were conducted to further examine the behavioral and neurochemical interactions of NT with DA in rats. Locomotor activity was assessed in doughnut-shaped photocell cages. Dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations were measured in nucleus accumbens and corpus striatum by HPLC with electrochemical detection. Methylphenidate (5 mg/kg IP) increased locomotor activity without affecting the DOPAC/DA ratio, an estimate of DA turnover. Neurotensin (30 µg IC) alone did not alter locomotor activity but did increase DA turnover in both brain regions. When methylphenidate and NT were given concomitantly there was an attenuation of both methylphenidate-induced increase in locomotor activity and NT-induced increase in DA turnover. Apomorphine (5 mg/kg SC) increased locomotor activity and decreased DA turnover. Neurotensin did not alter either the apomorphine-induced increase in locomotor activity or decrease in DA turnover. Studies utilizing a lower dose of apomorphine (0.1 mg/kg SC), which is believed to act preferentially on presynaptic DA receptors, also produced decreases in DA turnover. As previously reported, this dose of apomorphine decreased locomotor activity. Most interestingly, both the neurochemical and behavioral effects of this low dose of apomorphine were attenuated by IC NT.

These results provide further evidence that NT can modulate dopaminergic function. This occurs whether the resultant behavior is manifested as an increase or decrease in locomotor activity and suggests one action of NT is a presynaptic effect on DA neurons. However, the precise synaptic locus and mechanism of action of NT is still unknown.

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100.9 PINEAL GLAND INVOLVEMENT IN THE CONTROL OF THE MODULATORY EFFECTS OF OPIOID PEPTIDES ON SCHOOLING BEHAVIOR. M. KAVALIERS* (SPON: R. Shivers). Dept. of Zoology, University of Western Ontario, London, Ontario, Canada N6A 5B7

A number of physiological and pharmacological investigations have indicated that there are significant interactions between the pineal gland and opioid peptides. Previous studies have also suggested that social behaviors and behavioral interactions between individuals, including those of schooling behavior, can be affected by opioid mechanisms. In the present investigation the roles of pineal-opioid interactions in determining social behaviors were examined with schooling goldfish.

Intraventricular administration of β-endorphin (5.0 pg/g body weight) in goldfish significantly enhanced the cohesiveness and duration of schooling and decreased the latency of school formation. Higher doses of endorphin (15.0 pg/g) decreased schooling behavior. These opioid effects could be blocked and reversed by naloxone, with naloxone by itself decreasing schooling behavior. In addition, there was a significant day-night rhythm in schooling behavior and the effects of endorphin, with significantly lower responses evident at night. Pinealectomy eliminated these diurnal variations in schooling behavior and resulted in a significantly lower day-time propensity for schooling. Diurnal variations in schooling behavior could be established by administration of melatonin, a major pineal hormone. Pinealectomy also significantly reduced the effects of endorphin on schooling in both the day and the night. Prior or concurrent administration of melatonin reduced the effects of pinealectomy and resulted in endorphins maintaining significant effects on schooling. In addition, in intact fish a number of the effects of naloxone could be blocked by prior or concurrent administration of melatonin. These data show that the pineal gland is involved in mediating the effects of opioid peptides and suggest that pineal-opioid interactions and mechanisms may have a role in the regulation of schooling and social behaviors in general.

Supported by NSERC grant S222A1.

100.10 EVALUATION OF THE ANTIDEPRESSANT ACTIVITY OF MIF-I IN LABORATORY ANIMALS. R. Gordon*, G. Youreneff*, W. Diamantis*, J. L. Perhach Jr. and R. D. Sofia. Wallace Labs., Div. Carter-Wallace, Inc., Cranbury, NJ 08512.

MIF-I (Pro-Leu-Gly-NH_2) is a hypothalamic tripeptide which has been reported to potentiate dopa and antagonize oxotremorine-induced tremors in laboratory animals (Plotnikoff et al., Proc. Soc. Exp. Biol. Med. 140: 811, 1972). Clinically, MIF-I has been reported to have varying degrees of success in the treatment of Parkinson's disease (Kastin and Barbeau, Can. Med. Assoc. J. 107: 120, 1972). Furthermore, MIF-I at a very low dose (0.1 mg/kg, ip) has been shown to reduce passive immobility during swimming in rats (Kastin et al., Pharm. Biochem. Behav. 9: 515, 1978), a test sensitive to tricyclic antidepressant agents (Porsolt et al., Europ. J. Pharmacol. 47: 379, 1978). In addition, pilot studies in humans reported that MIF-I shows promise as an antidepressant (Ehrensing & Kastin, Arch. Gen. Psychiat. 30: 63, 1974 and Am. J. Psychiat. 30: 562, 1978). The purpose of these studies was to further explore the spectrum of antidepressant activity of MIF-I over a broad range of doses and included tests for the antagonism of tetrabenazine ptosis, yohimbine potentiation, isolation-induced fighting behavior, muricide behavior, swimming immobility, effects on pentobarbital hypnosis, locomotor activity and rotorod performance.

Test results showed that MIF-I (1.0 and 10.0 mg/kg, ip) partially antagonized tetrabenazine-induced ptosis in mice, whereas 0.01 - 10.0 mg/kg of the tripeptide failed to potentiate yohimbine lethality. Fighting behavior of isolated mice was reduced by 1.0 mg/kg, po, of MIF-I, but doses of 0.01, 0.1 and 10.0 mg/kg were ineffective. MIF-I (0.1 and 10.0 mg/kg, po) antagonized muricidal behavior up to 4 hours in 50% of the "killer" rats. Immobility following forced swimming was not significantly reduced by oral administration of MIF-I (0.1 - 10.0 mg/kg). Oral MIF-I (0.001 - 1.0 mg/kg) potentiated the hypnotic effects of sodium pentobarbital (45 mg/kg, ip) whereas ip administration of MIF-I had no effect on barbiturate hypnosis except for the 1.0 mg/kg, ip, dose which significantly reduced pentobarbital sleeping time in mice. Locomotor activity in mice was not significantly affected by MIF-I (0.1 and 1.0 mg/kg, po and ip). In addition, the tripeptide also demonstrated a low potential for neurotoxicity with an oral NTD_{50} value estimated at >400 mg/kg for the rotorod. Although MIF-I has been reported to possess activity in a few selected models of mental depression in animals, the data for these studies show a lack of dose-response effects and a narrow range of pharmacologic activity of MIF-I. This makes it difficult to ascribe antidepressant activity to this peptide in animal models.

100.11 CHANGES IN MORPHINE SELF-ADMINISTRATION AFTER EXPOSURE TO IONIZING RADIATION: EVIDENCE FOR THE INVOLVEMENT OF BETA-ENDORPHIN. G. A. Mickley, K. E. Stevens, G. A. White*, G. L. Gibbs*, G. H. Moore* and W. Deere*. Department of Behavioral Sciences and Leadership, USAF Academy, CO 80840 and Penrose Cancer Hospital, Colorado Springs, CO 80903.

When C57BL/6J mice are exposed to ionizing radiation they exhibit a locomotor hyperactivity and straub tail. These radiogenic behaviors are similar to those observed after an injection of morphine and they can be reversed by naloxone (Mickley, et. al, Soc. Neuro Sci. Abstr., 7:166, 1981). These recent findings suggest some involvement of endogenous opiates in radiation-induced behavioral change. The present experiments further investigated this hypothesis by observing changes in morphine self-administration after irradiation and by measuring radiogenic changes in the levels of beta-endorphin in brain and pituitary.

Under the presumption that the release of endogenous opiates would decrease the need for exogenously supplied morphine, we hypothesized that after radiation exposure morphine-experienced mice would self-administer less of the opiate. Male C57BL/6J mice had continuous access to two drinking flasks which contained either water or morphine in saccharine water. Mice were allowed two days baseline drinking and were then irradiated with 600 rads ^{60}Co. Liquid consumption was subsequently recorded for five days after exposure. Irradiated mice drank significantly less morphine than did sham-irradiated controls ($p < .025$, Mann-Whitney U). This decrease could not be entirely attributed to a generalized radiogenic hypodipsia or taste aversion.

In a second experiment, levels of the endogenous opiate beta-endorphin were measured in the brains and pituitaries of irradiated (1500 rads, ^{60}Co) or sham-irradiated, C57BL/6J mice. Irradiation immediately reduced ($p < .025$, Mann Whitney U) the quantity of beta-endorphin in the brains of the mice. The amount of the endogenous opiate increased with the passage of time after either radiation exposure or sham irradiation. However, within one hour, the pituitaries of the irradiated mice were significantly depleted of beta-endorphin ($p < .025$, Mann-Whitney U) as compared to controls. It may be the case that radiation stress causes the release of endogenous opiates from the brain and pituitary into the blood (for similar mechanism see J. Rossier, et. al., Nature 270:618, 1977). The data might also be explained by radiogenic changes in the molecular structure of the endorphin.

These results are consistent with the hypothesis that radiogenic behavioral changes may be due, in part, to the fluctuations of endogenous opiates.

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100.12 DIBUTYRYL-c AMP POTENTIATION OF APOMORPHINE EFFECT IS INHIBITED BY CAERULEIN, Everett H. Ellinwood, Jr., M.D. and Kenneth Rockwell, M.D.*, P.O. Box 3870 Duke University Medical Center, Durham, North Carolina 27710.

Both cholecystokinin octapeptide (CCK) and the structurally similar but more potent decapeptide, caerulein (CAR) have been reported to substantially inhibit methylphenidate stereotypy. Additionally, in the central nervous system, CCK has been found to reduce DA turnover in parts of the caudate and accumbens, and increase dopamine cell firing. In the periphery, CCK has a substantial interaction with cyclic- GMP and AMP.

To further assess these brain peptide-dopamine interactions, we tested the effects of intraventricular CAR on postsynaptic dopaminergic mechanisms by examining CAR interaction with apomorphine.

We found that CAR enhanced at lower doses and inhibited at higher doses the stereotyped behaviors induced by apomorphine. Dibutyryl-c AMP potentiates apomorphine in a very narrow dose range. Dibutyryl-c GMP in extensive dose ranging studies did not affect apomorphine behaviors. The potentiation of apomorphine behaviors by dibutyryl-c AMP is substantially inhibited by higher doses of CAR.

100.13 PHARMACOLOGICAL AND NEUROCHEMICAL INTERACTIONS OF ACTH AND THE MESOLIMBIC DA SYSTEM. J. S. Springer,* J. H. Hannigan, J. P. Ryan,* and R. L. Isaacson. Center for Neurobehavioral Sciences & Department of Psychology, SUNY-Binghamton, Binghamton, NY 13901.

Intracerebroventricular (icv) injections of ACTH induces enhanced grooming in rats (Gispen et al., Life Sciences 17: 645, 1975). This enhanced grooming can be altered by the pharmacological manipulation of the ascending dopaminergic (DA) system in nucleus accumbens (Cools et al., European J. Pharmac. 50: 265, 1978). Recently, we found that the intra-accumbens injection of ACTH induces excessive grooming at doses lower than required to see similar effects after an icv injection (Isaacson et al., in press, 1982).

This suggests a DA-ACTH interaction at the level of the nucleus accumbens. In this study we examined this interaction further in two experiments. In the first we studied the effect of intra-accumbens ACTH in animals with DA terminals in the nucleus accumbens lesioned by 6-hydroxydopamine. The second experiment was designed to determine the in vitro effects of ACTH on the K+-depolarized, Ca^{2+} dependent release of DA from synaptosomes prepared from either the nucleus accumbens or neostriatum.

In experiment one, rats were chronically implanted with bilateral cannulae into nucleus accumbens. After recovery, the rats received 25 mg/kg (ip) of DMI. One hour later, half of the rats received bilateral intra-accumbens 6-OHDA-HCl (4 µg/1.0 µl of saline and ascorbate). Ten days later all animals received bilateral intra-accumbens ACTH (0.5 µg/0.5 µl) and were tested for grooming as described by Gispen et al., 1975. The results indicate that the removal of the DA input to the nucleus accumbens significantly decreases the ACTH-induced excessive grooming.

In experiment two, synaptosomes were prepared as described by Hajos (Brain Res. 93: 485, 1975). Crude synaptosomes were incubated with either 0.1 µM apomorphine or 1.0 µM ACTH and release was determined as described by Babitch et al. (Life Sciences 24: 117, 1979). ACTH at a dose of 1.0 µM significantly decreases the Ca^{2+}-dependent release of DA in the nucleus accumbens, but produced less of an effect in the neostriatum.

This suggests that there is a DA-ACTH interaction in nucleus accumbens that may be important in the expression of some behaviors, and that there is a direct effect of ACTH on DA release from presynaptic terminals.

100.14 HIPPOCAMPAL LESIONS: ARE THE SECONDARY DOPAMINERGIC CHANGES FOLLOWING THE LESION RESPONSIBLE FOR THE DECREASE IN ACTH-INDUCED EXCESSIVE GROOMING? J. R. Hannigan, M. A. Balaz,* J. E. Springer,* J. P. Ryan,* and R. L. Isaacson. Center for Neurobehav. Sciences & Department of Psychology, SUNY-Binghamton, Binghamton, NY 13901.

Excessive grooming induced by intracerebroventricular (icv) administration of $ACTH_{1-24}$ is attenuated in rats with hippocampal lesions due to reduced sensitivity to the peptide (Elstein et al., Beh. Neur. Bio., 32: 248-251, 1981). Behavioral changes after hippocampal damage can be due to altered dopamine (DA) systems in nucleus accumbens (NA), as reported by Reinstein et al. (Pharmac. Biochem. Beh., in press, 1982). The reduced sensitivity to ACTH also may be mediated by altered DA systems in NA. In fact, it has been shown that injection of drugs that influence DA systems into NA will interfere with icv ACTH-induced excessive grooming (Cools et al., Eur. J. Pharmac. 50: 265-268, 1978) and intra-accumbens ACTH will induce excessive grooming at lower doses than icv ACTH (Isaacson et al., in press, 1982a).

Recently we proposed that excessive grooming may reflect at least two neural substrates. The pharmacological data related to peptide-induced grooming suggest separate but interactive DA-sensitive and opiate-sensitive components. The present study was designed to further characterize these systems in the hippocampally lesioned rat.

Rats were prepared with sham, cortical or hippocampal lesions by aspiration and at the same time were implanted with cannula into NA and the interventricular foramen. Starting one month after surgery, the rats received bilateral injections of either saline (.5 µl) or the DA agonist 3,4-dihydroxyphenylamino-2-imidazoline (DPI; 2.5 µg/.5 µl) into NA. Twenty min later, all rats received 1 µg/µl $ACTH_{1-24}$ icv and were measured for grooming, rearing, and locomotion for 60 min.

Sham and cortical controls demonstrated excessive grooming which was reduced by DPI. As expected, the hippocampal lesions reduced the levels of grooming relative to controls with intra-accumbens saline. Injections of DPI in the hippocampal group significantly increased the amount of grooming relative to saline injected hippocampals.

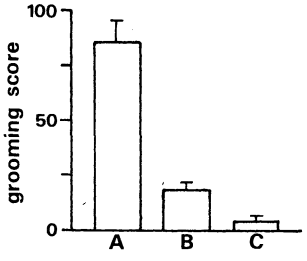
In a second study, injections of $ACTH_{1-24}$ directly into NA of lesioned rats were less effective in inducing excessive grooming in comparison to control lesioned rats. The NA is a likely site of action for ACTH (see Springer et al., Neurosci. Abst. 8: 1982) but it may not be sensitive to opiate blockade (Ryan et al., in preparation). It appears then that the effect of both the peptide and the hippocampal lesion on grooming is mediated through DA systems. These results support the idea that both limbic and neuropeptide mechanisms modulate the basal ganglia activities.

100.15 PEPTIDE-INDUCED EXCESSIVE GROOMING BEHAVIOR: THE ROLE OF OPIATE RECEPTORS. V.J. Aloyo, B. Spruijt*, H. Zwiers* and W.H. Gispen*. Division of Neurobiology, Institute of Molecular Biology, and Rudolf Magnus Institute of Pharmacology, State University of Utrecht, Utrecht, The Netherlands.

Intracerebralventricular (ICV) administration of adrenocorticotropic hormone (ACTH) and several opioid peptides such as β-endorphin (β-E, βLPH_{61-91}) and $dynorphin_{1-13}$ (dyn) induces excessive grooming in the rat (Gispen and Isaacson, Pharmac. Ther. 12, 209, 1981). The ability of the specific opioid antagonists, naloxone and naltrexone, to block this peptide-induced behavior indicates the involvement of an opioid-sensitive neural process. However, it is not yet known whether the opioid receptors are the primary site of interaction with the peptides or are involved later in the chain of events leading to grooming behavior. It has previously been postulated (Zwiers *et al.*, Life Sci. 28, 2545, 1981) that the basic residues in these peptides are involved in the induction of excessive grooming.

To test whether the affinity for the opiate receptor is a prerequisite for the induction of grooming, we have evaluated the ability of non-opioid analogs of dynorphin and β-endorphin to induce grooming. The non-opioid peptides, des-tyrosine dynorphin (dT-dyn, $dynorphin_{2-13}$) and des-tyrosine β-endorphin (dTβ-E, βLPH_{62-91}) are both capable of inducing excessive grooming after ICV administration. The figure shows that the $dynorphin_{2-13}$-induced grooming is blocked by the prior administration of either naloxone or haloperidol as has previously been shown for opioid peptides as well as non-opioid peptides such as ACTH. Similar results have been obtained for dTβ-E.

From these results we conclude that peptide-opioid receptor interaction is not the primary event in peptide-induced grooming.



Rats were injected (s.c.) with saline (A), 1 mg/kg naloxone (B) or 0.2 mg/kg haloperidol (C) 5 min prior to the ICV administration of 1 µg dT-dyn.

101.1 DOES BLOCKADE OF GABA UPTAKE AFFECT THE TIMECOURSE OF GABA-EVOKED CONDUCTANCE CHANGES? Stephen J. Korn and Raymond Dingledine, Dept. of Pharmacology, Univ. of North Carolina, NC 27514.

Biochemical studies have demonstrated the existence of gamma-amino butyric acid (GABA) uptake systems in brain tissue. These uptake processes are hypothesized to function as the mechanism for termination of transmitter action at GABAergic synapses. Physiological studies in vivo, however, have failed to provide support for this hypothesis. We are examining this hypothesis for recurrent inhibitory synapses in the in vitro hippocampal slice preparation. Intracellular recordings were made from pyramidal cells in hippocampal region CA1. The slices were bathed in 1 μM TTX to allow measurement of membrane potential responses to iontophoresed GABA without interference from Na mediated action potentials. Hyperpolarizing current pulses (0.25 to 0.5 nA) were injected into the cell through the recording electrode every 150 to 200 msec to measure the cell's input resistance. Iontophoresis of GABA into the cell layer resulted in a decreased input resistance accompanied by a hyperpolarization of the cell. When iontophoresed into the dendritic region, GABA decreased the input resistance and depolarized the cell. The magnitude and time-course of the GABA-evoked conductance change were monitored by measuring the change in input resistance over time. The decay of the GABA response has two phases, an early, nonlinear, "preexponential" phase and a later "exponential" phase. The exponential decay time cnstant and the magnitude of the GABA response increased with increasing doses of GABA. Nipecotic acid (1 mM), an inhibitor of GABA uptake into neurons, prolonged both decay phases of the GABA response, increased the maximum conductance change, and increased the magnitude of the membrane potential change evoked by GABA. Reduction of extracellular Na from 155mM to 25mM, a procedure which also inhibits GABA uptake, also prolonged both decay phases of the GABA response. Both hyperpolarizing and depolarizing responses to GABA were prolonged and potentiated by nipecotic acid. In the presence of nipecotic acid, doses of GABA which normally elicited only a hyperpolarizing response elicited a biphasic response (hyperpolarizing, then depolarizing), suggesting that uptake blockade resulted in diffusion of GABA from the somatic to the dendritic region of the cell. These results suggest that the hippocampal pyramidal cell region, known to contain recurrent inhibitory synapses which use GABA as a neurotransmitter, contains GABA uptake mechanisms that temporally and spatially limit the response to exogenously applied GABA. The effect of uptake blockade on the timecourse of synaptically released GABA is currently under investigation.
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101.2 SELECTIVE ACTIONS OF PENTOBARBITAL ON HIPPOCAMPAL GRANULE AND PYRAMIDAL NEURONS STUDIED IN VITRO. M.B. MacIver*, B.H. Bland* and S.H. Roth. Dept. of Pharmacology, University of Calgary, Calgary, Alberta. T2N 1N4.

Barbiturates have been shown to produce selective effects on different types of invertebrate neurons (Johnston, D., Neurosci. Letters, 10: 175, 1978), and on heterogenous groups of cultured spinal neurons (Barker, J.L. & Ransom, B.R., J. Physiol., 280: 355, 1978). The purpose of the present study was to investigate whether selective effects are produced by pentobarbital on the tri-neuronal pathway from dentate granule cells to CAI pyramidal neurons in the rat and guinea pig hippocampal formation. Evoked field potentials and single unit discharge activities were recorded from 300 to 400 μm thick hippocampal brain slices maintained in vitro. Bipolar metal electrodes were placed on perforant path, mossy fiber and Schaffer collateral fibers and were used to stimulate these pathways (5.0-15V, 0.01-0.05 mSec). Evoked field potential responses (4.0-6.0 mV, peak to peak) and single unit discharge activities were recorded using glass electrodes (4M NaCl, 4-15 MΩ) placed in the cell body layers of dentate granule cells, CAIII, and CAI pyramidal neurons. The slices were supported on top and bottom by nylon mesh and were submerged under 2mm of solution. Pre-oxygenated (95% O_2, 5% CO_2) and warmed artificial CSF and drug containing solutions were continuously perfused through the tissue chamber (4ml/min; 32°C). Low concentrations of pentobarbital (0.1 to 0.4 mM) produced an enhancement of CAIII and CAI pyramidal neuron evoked field potential responses (mossy fiber → CAIII; Schaffer collateral → CAI). This enhancement was observed as an increase in amplitude of both the field EPSP and population spike. Evoked single unit discharge recordings revealed that the increased population spike amplitude was due to increased discharge frequency and to recruitment of additional neurons. Similar concentrations of pentobarbital produced depression of the perforant path to dentate granule cell evoked field potential (complete depression at 0.4 mM). This depression was due primarily to a reduction of the population spike amplitude, while the field EPSP amplitude was only slightly reduced by concentrations less than 0.5 mM. Concentrations lower than 0.1 mM produced only slight and inconsistent effects on granule and pyramidal neurons. Pentobarbital (0.5 to 1.0 mM) produced complete depression of granule, CAIII, and CAI field potentials and discharge activity. These results suggest that different neurons are affected in a selective manner by pentobarbital and that more than one mechanism of action may be involved.

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101.3 SEPTAL MODULATORY ACTION IS DIMINISHED BY HEMICHOLINIUM-3 (HC-3). M. Glavinović, K. Krnjević and N. Ropert, Depts. Research in Anaesthesia & Physiology, McGill University, Montréal, Québec.

There is strong evidence for the cholinergic nature of the septo-hippocampal pathway. 1. The facilitatory action of medial septal trains on commissural- or fimbrial-evoked population spikes in the CA1 region is similar to the action of acetylcholine (ACh). 2. It is also reduced by atropine or scopolamine and 3. It is enhanced by local applications of physostigmine (Krnjević, K. & Ropert, N., 1981, Can. J. Physiol. Pharmacol., 59: 911). As a further test, we have studied the effects of HC-3 - a known choline uptake blocker, which reduces ACh synthesis - on the septo-hippocampal action.

The experiments were performed on urethane anaesthetized rats whose medial septum and hippocampal commissure were stimulated stereotaxically with bipolar electrodes. Low frequency (< 1 Hz) commissural stimulation evoked a positive wave in CA1 stratum pyramidale, but applications of ACh or short duration (100 ms) high frequency (50-100 Hz) septal trains preceding the commissural shock induced population spikes. During longer trains, lasting from 0.5-5 min population spikes were first enhanced and then depressed. Unilateral intraventricular injections of HC-3 (7.5-15 μg in 1 μl) reduced the efficacy of septal trains while the responses to ACh were generally unaltered. When the effect was established the hippocampus was removed and its ACh content subsequently measured. In HC-3 treated preparations, which after prolonged tetanic stimulation showed decreased efficacy of septal trains, the ACh content was reduced to on the average 1/6 of the normal level.

A complication of the HC-3 administration is that it was followed by a slow increase in the amplitude of population spikes evoked at a constant intensity of commissural stimulation (over an hour or more). In other experiments, we found that iontophoretic applications of HC-3 in the hippocampus also produce a slow facilitation of population spikes. This effect is in keeping with previous observations that HC-3 has an excitatory action on ACh-sensitive neocortical neurons in cats (Krnjević et al., 1971, J. Physiol., 215:223). Since choline has been shown to exert a direct excitatory effect on cortical neurons (Krnjević, K. and Reinhardt, W., 1979, Science, 206:1321), the excitatory action of HC-3 can be accounted for most simply by a gradual accumulation of extracellular choline, resulting from diminished choline uptake by cholinergic terminals. Our observations provide further support for the cholinergic nature of the septo-hippocampal pathway.

Supported by the Medical Research Council of Canada.

101.4 EFFECTS OF AMMONIUM IONS ON SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS IN VIVO. J.-L. Bossu* and Y. Théorêt* (SPON: N. Lake). Depts. of Anesthesia Research and Pharmacology and Therapeutics, McGill Univ., Montreal, Canada H3G 1Y6.

Previous results obtained in the hippocampal slices indicate that ammonium (2-5 mM) depresses synaptic transmission from perforant path to dentate granule cells, mossy fibers to CA3 pyramidal cells, and Schaffer collaterals to CA1 pyramidal cells, without a significant decrease of antidromic conduction in regions studied (Théorêt, Y. et al., Proc. Can. Fed. Biol. Soc., 24:239, 1981). It seemed therefore important to confirm these findings on in vivo preparation. Stereotaxic stimulation of fibers running in the fimbria generated field potential responses in CA1 and CA3 pyramidal cells. Intraventricular cannulae were implanted and the lateral ventricle was perfused with osmotically balanced artificial CSF containing either ammonium chloride or ammonium acetate in concentrations ranging from 10-30 mM. Ammonium induced a reversible depression of orthodromic field potential responses in pathways tested. The magnitude of the depression and the rate with which it developed were concentration dependent. Antidromic responses elicited in CA3 pyramidal cells were relatively unaffected, indicating that neither axonal conduction nor electrical excitability of the postsynaptic neurons were affected by ammonium. In some experiments, a transient increase in the size of population spike concomitant to a decrease in the late positivity was observed prior to the depression, suggesting a period of disinhibition. This possibility is being further investigated. Preliminary experiments indicate that unit activity evoked by iontophoretically applied glutamate was not depressed by ammonium. These data are compatible with the suggestion that ammonium depresses glutamate release evoked by high potassium in the hippocampal tissue (Hamberger, A. et al., J. Neurochem., 33: 1295, 1979), presumably by inhibiting its synthesis. It is suggested that the observed decrease of excitatory synaptic transmission may be the tentative mechanism contributing to coma and other symptoms of central nervous system depression induced by high ammonium concentrations.

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101.5 THEOPHYLLINE MODULATES SYNAPTIC EFFICACY AND SHORT-TERM PLASTICITY IN THE RAT IN VITRO DENTATE GYRUS. Linda K. Simmons. Psychology Department, Harvard University, Cambridge, Mass. 02138.

The effects of theophylline, a phosphodiesterase inhibitor, on the activity of the perforant path-granule cell synapses were examined in the rat hippocampal slice. Theophylline (1-3 mM) consistently enhanced the extracellularly recorded population synaptic response (p-EPSP). Since the afferent volley remained unchanged and there was no indication of a postsynaptic hyperpolarization that would produce larger p-EPSPs during the theophylline induced enhancement, it is proposed that this drug enhances synaptic efficacy.

Perfusion with cyclic AMP analogues (dibutyryl cAMP and 8'-bromo cAMP) or stronger phosphodiesterase inhibitors (RO 20-1724 and ZK 62711) did not mimic the effects observed with theophylline, suggesting that the enhancement is unrelated to manipulation of tissue cyclic AMP levels. Combination adenosine/theophylline incubation experiments demonstrated that theophylline enhances synaptic efficacy by a mechanism that does not solely involve blocking the depressant effects of circulating adenosine.

Investigators have determined that theophylline can be a potent stimulator of intracellular Ca^{++}. Also, the efficacy of synaptic transmission is largely dependent upon the levels of Ca^{++} in the extracellular medium. Studies were carried out to characterize the Ca^{++} dependence of two short-term forms of synaptic plasticity at the perforant path-granule cell synapses: paired pulse facilitation and depression. The experimental paradigm consisted of presenting equal intensity paired stimulus pulses to the perforant path fibers with various interpulse intervals (7-1000 msec) and analyzing the degree of enhancement or depression of the second (test) response relative to the first (conditioning) p-EPSP. In high (2.5 mM) Ca^{++} Ringers solution the test synaptic responses were uniformly depressed relative to the conditioning responses at all interpulse intervals. In low (1 mM) Ca^{++} the test responses were facilitated.

When 3 mM theophylline was added to either of the two Ca^{++} concentration media, the test/conditioning response ratios were significantly smaller than the corresponding ratios measured in the absence of theophylline. Thus, theophylline caused an increase in depression or a decrease in facilitation, depending upon the Ca^{++} concentration of the bathing medium. Given the above described Ca^{++} dependence of short-term plasticity, theophylline manipulated short-term plasticity in a manner which suggests that the drug increases Ca^{++} availability at the dentate gyrus synapses.

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101.6 PHARMACOLOGICAL INVESTIGATION OF PAIRED-PULSE POTENTIATION AT THE HIPPOCAMPAL PERFORANT PATH SYNAPSE. Eric W. Harris, Thomas H. Lanthorn and Carl W. Cotman. Deptartment of Psychobiology, Univ. of Calif. Irvine, Irvine, CA 92717.

Synaptic transmission along the perforant path (from entorhinal cortex to the hippocampal dentate gyrus) exhibits several properties such as potentiation and habituation. There has been considerable electrophysiological description of the conditions under which these phenomena will occur, but there have not been any studies making use of recent findings concerning perforant path pharmacology. In this report we use the in vitro hippocampal slice preparation to study the simplest synaptic dynamics, the response to paired stimuli applied to the perforant path.

In response to paired-activation, the extracellular recorded negative wave evoked by activation of the lateral portion of the perforant path exhibits potentiation, whereas the synaptic wave associated with the medial portion of the perforant path shows a habituation-like decrement. Recent work in our laboratory has shown that the compound 2-Amino-4-Phosphono-Butyric Acid (APB) selectively and reversibly antagonizes responses along the lateral perforant path in micromolar concentations. We now report that addition of APB to the medium bathing a submerged hippocampal slice not only decreases the first of a pair of responses as expected, but also causes an increase in the percent paired-pulse potentiation at a given stimulus intensity (40 msec inter-stimulus interval, 10 sec inter-pair interval). This effect is dose-dependent, reverses upon drug wash-out, and does not appear to involve any change in the time course of potentiation. Other pharmacological manipulations that decreased lateral perforant path responses (addition of mM D-α-Amino-Adipic Acid; increasing extracellular $[Mg^{++}]$; decreasing extracellular $[Ca^{++}]$) did not have similar effects on potentiation.

A given dose of APB had a proportionately smaller effect on the second (test) response than on the first (conditioning) response, although the absolute magnitude of antagonism of each was similar. Analysis of dose/response curves revealed that the conditioning and test responses are pharmacologically different: the first response is almost entirely "APB-sensitive", whereas the test response has an additional component that is less sensitive to reduction by APB.

This study reveals a previously unsuspected complexity in the response to stimulation of the perforant path, and will aid in evaluating mechanisms of potentiation.

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101.7 NON-CHOLINERGIC, EXCITATORY TRANSMISSION IN RAT NEOSTRIATUM. Gary Cordingley and Forrest Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, Maryland 20852

The synaptic pharmacology of an excitatory pathway in the neostriatum was studied using electrophysiological techniques in the tissue slice. 400-500 μm thick parasagittal sections from the anterior-superior quadrant of the rat caudate-putamen were submerged in a flow-through chamber containing artificial CSF at 33-35°C, saturated with 95% O_2-5% CO_2. Bipolar, 40 μm dia., platinum-iridium, stimulating electrodes with 80-120 μm tip separation were advanced into the tissue. Extracellular potentials were recorded within 225-425 μm of the cathode, with glass microelectrodes containing 3 M NaCl (0.5-6 MΩ). A second microelectrode minimized the stimulus artifact, through differential recording. A series of two negative potentials (N-1 and N-2) followed stimuli 6-86 V in amplitude and 0.1 ms in duration. With stimulus intensity adjusted for maximal N-2, N-1 was 0.3-1.2 mV with a peak latency of 1.1-2.2 ms, and N-2 was 0.1-0.9 mV at 3.0-4.4 ms. Unitary spike discharges were often recorded at or near the peak of N-2, and less often, near the peak of N-1. N-1 and N-2 were abolished by 10 mM procaine, indicating a neural origin for both. Only N-2 was abolished (reversibly) by low-calcium (0.3 mM) or high-magnesium (8.0 mM) solutions, indicating a synaptic step in its generation. To identify the endogenous transmitter, slices were superfused with solutions containing transmitter antagonists. Acetylcholine antagonists d-tubocurarine (30 μM), mecamylamine (0.1 mM) or atropine sulfate (0.1 mM) failed to alter the potentials. By contrast, Misgeld et al. (Exp.Br.Res. 34:575, 1979 and 39:401, 1980) reported that nicotinic antagonists block N-2, using coronal slices of neostriatum in an interface chamber with surface-stimulating electrodes. We also found that dopamine antagonists L-sulpiride (0.3 μM) and fluphenazine hydrochloride (10 μM) were ineffective. Among putative antagonists of excitatory amino-acid transmitters, however, baclofen, DL-2-amino-4-phosphonobutyric acid (APB), and L-glutamic acid diethyl ester blocked N-2 with IC50's of 0.8 μM, 1.4 mM and 10 mM, respectively. Complete blockade was produced by 3 μM baclofen and 3 mM APB; the blockade was reversible. Our results suggest that N-1 results from direct activation of fiber tracts, while N-2 is a population spike mediated by excitatory synapses using a glutamate-like transmitter.

L-sulpiride, fluphenazine hydrochloride and baclofen were gifts from the Ravizza, Schering and Ciba companies, respectively. G.C. is a Pharmacology Research Associate of the National Institute of General Medical Sciences.

101.8 COCAINE: COMPARATIVE UPTAKE INHIBITION OF DOPAMINE IN EXTRAPYRAMYDAL AND LIMBIC SYSTEMS. M. G. Hadfield and E. A. Nugent*. Neurochemistry Research Lab., Section on Neuropathology, Dept. of Pathology, Med. Coll. Va., Va. Commonwealth U., Richmond, Va. 23298.

Cocaine is a potent competitive inhibitor of catecholamine uptake. That is, it vies with neurotransmitter for uptake carrier sites but does not alter transport velocity.

In the present study, we compared cocaine inhibition of dopamine (DA) uptake in synaptosomes obtained from the prefrontal cortex, olfactory bulbs and striatum of male ICR mice. The cocaine concentration varied from 0.1 μM to 10 μM and the DA concentration was 0.2 μM. 0.01 μM desmethylimipramine (DMI) was added to prevent DA uptake into norepinephrine terminals.

At the lower concentrations of cocaine, little difference in uptake was noted (4-11% inhibition in all systems at 0.1 μM and 19-29% inhibition at 1 μM). But at concentrations greater than 10 μM, only the striatum showed very marked to virtually complete DA uptake inhibition (86% at 10 μM, 92% at 100 μM, 99% at 1 μM and 99% at 10 μM cocaine). At these higher concentrations, DA uptake plateaued in the limbic system (40-54% in prefrontal cortex and 35-48% in olfactory bulbs). However, when % inhibition was plotted vs. the -log of cocaine concentration, the IC_{50} values were not that dissimilar (≅4 μM for striatum, ≅3 μM for olfactory bulbs and ≅1 μM for prefrontal cortex).

These results may be due to a number of factors: (i) The prefrontal cortex and olfactory bulbs are relatively poor in DA terminals as compared with the striatum. Exogenous DA uptake may have occurred in neurotransmitter terminals not affected by cocaine or DMI. (ii) There may be non-specific uptake of DA by structures that are more abundant in the prefrontal cortex and olfactory bulbs than in the striatum. (iii) DA uptake receptors may be different in the limbic system than in the extrapyramidal system. This is consistent with the differential effects of certain neuroleptics, etc., on limbic and extrapyramidal DA activity as reported by others.

If the cocaine effect reported here is due, at least in part, to genuine differences in DA neurotransmission in the limbic and extrapyramidal systems, then it may help us define molecular mechanisms responsible for those differences.

101.9 NORADRENALINE MEDIATED INHIBITORY POSTSYNAPTIC POTENTIALS IN LOCUS COERULEUS NEURONS. J. T. Williams,* T. M. Egan,* R. A. North and G. Henderson. Neuropharmacology Laboratory, Department of Nutrition and Food Science, M.I.T., Cambridge, MA 02139.

Intracellular recordings were made from rat locus coeruleus (LC) neurons in a slice preparation. The slice was completely submerged in artificial cerebrospinal fluid at 37°C. Drugs were applied by perfusion and by pressure ejection into the solution just above the slice. It was possible to record from single cells in slices maintained in vitro for periods of up to 14 hrs. Most cells showed spontaneous action potentials (mean amplitude 82.5 mV; duration 1.5 ms) throughout the recording period. Electrical stimulation with bipolar tungsten electrodes in the area of the LC resulted in an excitatory synaptic potential (e.p.s.p.) followed by an inhibitory synaptic potential (i.p.s.p.). The amplitude of the i.p.s.p. was graded with the strength of stimulation; the amplitude declined with repetitive stimulation at frequencies greater than 0.1 Hz. Both i.p.s.p. and e.p.s.p. were completely abolished in calcium-free, high magnesium solutions. The ionic mechanism of this i.p.s.p. is probably due to an opening of potassium channels since (1) the reversal potential was at the potassium equilibrium potential (E_K) and (2) agents which decrease potassium conductance (eg. Ba) also blocked the i.p.s.p. The same ionic mechanism has been found for the hyperpolarization induced by exogenously applied noradrenaline (NA). In addition, perfusion of the NA uptake inhibitor desmethylimipramine (DMI) potentiated both the i.p.s.p. and the hyperpolarization induced by NA. Finally, perfusion with α_2-antagonists (phentolamine and yohimbine) antagonized both the i.p.s.p. and the NA induced hyperpolarization. The results support the suggestion that the excitability of LC neurons might be controlled by recurrent collateral inhibition, and demonstrate a potassium ion mediated i.p.s.p. in the LC.

102.1 PHENCYCLIDINE EFFECTS MEASURED INTRACELLULARLY IN HIPPOCAMPAL CA1 CELLS ARE DOSE DEPENDENT. P.W. Kujtan*, P.L. Carlen. Institute of Medical Science, Depts. of Medicine and Physiology, University of Toronto. Addiction Research Foundation Clinical Institute and Playfair Neuroscience Unit, Toronto Western Hospital, Ontario, Canada.

Phencyclidine (PCP) abuse is rapidly approaching epidemic proportions. Brain energy metabolism studies (Meibach et al. 1979) as well as binding studies (Zukin and Zukin, 1979) have implicated the hippocampus as a major site of action. However, the basic mechanisms of PCP effects are as yet unknown. For this reason, the guinea pig transverse hippocampal slice was used as a model to investigate PCP action.

Male guinea pigs, 300 mg. in weight, were sacrificed, the left hippocampus removed, and microtomed into 400 um transverse slices, which were placed into a recording chamber at 35°C. Micropipettes filled with 3M potassium acetate (resistance 80-180 M) were used for intracellular recording. The Schaeffer collaterals were stimulated using monopolar tungsten tip electrodes. PCP was dissolved in artificial CSF and applied by either bath perfusion or pressure ejection.

Perfusion of 2.0 uM PCP increased the spontaneous activity in 7 of 8 cells. This was accompanied by a decreased IPSP size, increased input resistance, as well as an increased threshold as measured by current pulse injection. Three cells showed spontaneous burst discharges 3-5 seconds in duration. Extracellularly measured orthodromically evoked field potentials were also seen to be increased at this dose. Pressure ejection of 2.0-4.0 uM PCP resulted in more variable responses. Of 14 cells tested, spontaneous activity was unaffected in 7 cells, increased in 5, and decreased in 2 cells. Burst discharges were also seen in 4 cells. Perfusion of 0.4→2mM PCP for the initial few minutes hyperpolarized the membrane and inhibited spontaneous activity. This was followed by large depolarizing shifts, 3 and 4 spike bursts, prolonged (3-20 second) depolarizations with intense spiking followed by quiescence, and a prolongation of spike width. Extracellularly evoked field potentials also showed depressed responses at these high doses. Pressure ejection of 0.4→2mM PCP resulted in a transient decrease in activity accompanied by membrane hyperpolarization.

These preliminary experiments suggest several mechanisms of action depending on the dose used, and might also be related to the clinically observed mixture of excitatory and inhibitory effects of PSP intoxication.

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102.2 LACK OF BENZODIAZEPINE RECEPTOR ALTERATION AFTER ACUTE OR SUB-CHRONIC BENZODIAZEPINE ANTAGONIST (RO 15-1788) ADMINISTRATION IN MICE. N.M. SPIRT, M. ZANKO, G. BAUTZ AND R.A. O'Brien (SPON: R. MANGANO). DEPT. OF PHARMACOLOGY II, HOFFMANN-LA ROCHE INC., NUTLEY, N. J. 07110.

CNS benzodiazepine (BZ) receptors are part of a macromolecular membrane complex which modulates changes in GABA neurotransmission and chloride ion channel fluxes. The BZ receptor part of this complex has been shown to undergo very rapid changes in either number (Sci. 202;892, 1978) or affinity (PBB.10;809, 1979) following seizures of drugs respectively. Also, acute diazepam administration produced increases in BZ number (Eur. J. Pharmacol. 59;159, 1979). Since neurotransmitter receptor systems (e.g. dopamine) respond to the administration of a receptor blocker or antagonist (e.g. haloperidol) by an icrease in receptor number as a compensatory response, we investigated this regimen in mice treated with the BZ receptor antagonist RO 15-1788. An increase in receptor number, if compensatory responses were active, might also indicate indirectly the presence of an endogenous BZ agonist operating at these receptor sites. We tried a short treatment regimen since previous reports indicated that BZ receptors changed number or affinity very rapidly (see above refs.) Mice (Charles River, 14-15 gms, female, CD-1 Kingston) were injected intraperitoneally 3 x a day for three days with 50 mg/kg RO 15-1788 in acacia vehicle. A second group of mice were injected with only one 50 mg/kg dose. Mice from both groups was killed at varying times after the last dose (.25, .5, 1,2,4,6, 24 hrs.) Prior to receptor binding, the brains were washed extensively to remove any residual bound drug. Total specific binding indicated a change (+ 30%) in only one treated group, those mice killed 6 hrs. after the one acute dose of RO 15-1788. To determine the significance of this observation a second series of experiments were done to analyze the change seen at 6 hrs. using a saturation isotherm and scatchard analysis paradigm. Twenty control and RO 15-1788 treated mice were killed 6 hrs. after dosing. Both the antagonist-treated and vehicle controls displayed the same K_D (5.5 nM) and B_{max} (1.6 pMoles/mg protein). Thus, unlike agonist treatment, the antagonist RO 15-1788 did not alter BZ receptors significantly under the conditions tested. Such results may indicate that (1) BZ receptors do not respond to antagonist treatment in a manner similar to neurotransmitter receptors, (2) there is no endogenous BZ ligand or (3) the treatment regimen used in this study was not adequate to detect sensitive changes in BZ receptor alteration.

102.3 A COMPARISON OF THE EX VIVO BINDING OF FLURAZEPAM AND ITS METABOLITES WITH TEMAZEPAM AND TRIAZOLAM. G. BAUTZ, N.M. SPIRT, W.D. HORST, M. ZANKO AND R. A. O'BRIEN. DEPT. PHARMACOLOGY II, HOFFMANN-LA ROCHE, INC., NUTLEY, NJ 07110.

In a previous study of ex vivo benzodiazepine binding, we found that brain receptor binding more closely correlated with clinical effects than did the half-life of elimination of the drug (Soc. for Neurosci. Abst. 7,865,1981). In this study we investigated the ex vivo binding time course of flurazepam and its metabolites, N-desalkylflurazepam and N-1-hydroxyethylflurazepam and compared their interaction with brain benzodiazepine receptors with two new hypnotics, temazepam and triazolam. Since hypnotics of this type might be used on an acute basis as well as on a chronic regimen, we sought to determine the brain receptor binding profile of an acute dose and possibly gain information on the probability of these drugs or metabolites having a prolonged combination with brain receptor sites. This information could be useful in predicting the likelihood of residual or "morning after" side effects in instances where only one evening drug dose is administered. After a dose-ranging study was conducted by intravenous administration of the above compounds to male Charles River Sprague Dawley rats (170-190 grams), IC_{50} (mg/kg) values were determined and these doses were injected to a second series of rats and the time course of brain receptor binding measured over a 24 hour time period. The slopes of the disappearance curves representing dissociation of drug from brain receptor sites was comparable for each drug as well as for the major metabolites of flurazepam. While initial doses of flurazepam (3 mg/kg), triazolam (.1 mg/kg) and temazepam (3 mg/kg) were used, each drug had produced less than 10% inhibition of ^{3}H-diazepam binding by 60 minutes after the time of injection. In a separate experiment, the flurazepam metabolites produced no residual inhibition of binding 24 hrs. after drug administration and this was in spite of the fact that at one half min. after injection there was 90% inhibition of ^{3}H-diazepam binding compared to vehicle controls. We conclude that, after acute equieffective doses of flurazepam, triazolam or temazepam, the amount of residual drug bound at benzodiazepine receptors will produce comparable inhibitory effects and that predictions of drowsiness on the day following an acute drug dose based on half-lives, may not be accurate.

102.4 BENZODIAZEPINE RECEPTOR BINDING: EFFECT OF FLUROTHYL INDUCED SEIZURES. J. Guarino*, M.Gay*, N. Boisse. (SPON: C. Kornetsky). Section of Pharmacology, Northeastern University, Boston, MA 02115

Flurothyl (hexafluorodiethylether) induced seizures are currently being used as a sensitive measure of CNS excitability to develop a low dose benzodiazepine dependence model. Since benzodiazepine receptors may be involved in the induction and expression of this dependence and they have been reported to increase in number following electroshock and pentylenetetrazole induced seizures (Paul and Skolnick, Sci.202:892-894, 1978), the effects of flurothyl seizures on benzodiazepine binding characteristics were explored. Flurothyl seizures were induced by inhalation as described by Adler (Arch. int Pharmacodyn. 170(1):12-20, 1967). A rat was placed in a 3 liter glass chamber and flurothyl (10% in ethanol) was delivered at a controlled rate of 0.1 ml/min. Typically the onset of clonic seizure with loss of posture occurred at 7 to 8 minutes in controls. 3H-flunitrazepam binding was evaluated at 0, 15, 30 and 60 minutes post-seizure by a modification of the method of Mohler and Okada (Sci.198:849-851, 1977). Binding of 3H-flunitrazepam to P2 fractions isolated from forebrain was carried out in an incubation volume of 200 ul at 0.1-26 nM 3H-flunitrazepam at 0°C for 30 minutes. K_D and Bmax were determined by Scatchard analysis. Controls showed a K_D of 1.52 (± 0.11 S.E.) nM and a Bmax of 878 (± 21 S.E.) fmole/mg protein. Flurothyl seizure produced no significant change in K_D or Bmax. However, single point analysis at 1.6 nM 3H-flunitrazepam (ca. K_D) showed a significant decrease ($P < .02$) in the amount of 3H-flunitrazepam bound only at 0.25 hrs after the seizure, 305 (± 17 S.E.) compared to controls, 380 (± 22 S.E.) fmoles/mg protein. Flurothyl induced seizures thus lead to a slight but significant decrease (20%) in specific binding of 3H-flunitrazepam which was observable at a ligand concentration near the K_D but was not observed in the kinetic constants derived from Scatchard analysis. The difference in the direction of change in the amount of specific binding observed between flurothyl seizures and electroshock or pentylenetetrazole seizures may reflect mechanistic differences between these seizure states or the patterns of seizure activity induced. (Supported by Northeastern University Research and Scholarship Development Fund).

102.5 EFFECT OF 6-SUBSTITUTED DERIVATIVES OF 2-AMINO-6-METHYLTHIOPURINE ON BRAIN SPECIFIC BENZODIAZEPINE RECEPTOR. S. C. Sung. Division of Neurological Sciences, University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

We have previously reported the effect of various purine derivatives on [^{3}H]diazepam binding to rat brain membranes. Among those analogs, 6-methylthioguanine was found to be most potent, inhibiting competitively the specific binding of [^{3}H]-diazepam with a K_i value of 16 μM. The purpose of this study was to compare the effect of various derivatives of 6-methylthio-guanine to displace [^{3}H]diazepam binding to rat brain membranes. Two hydrophobic derivatives, namely 6-benzylthioguanine (or 2-amino-6-benzylthiopurine) and 6-heptyldithioguanine (or 2-amino-6-heptyldithiopurine) were found to be more potent than 6-methyl-thioguanine; K_i value for both being approximately 6 μM. Contrary to 6-methylthioguanine which caused the same degree of inhibition either with brain homogenate or with membrane preparation, 6-heptyldithioguanine was more active with membrane than with homogenate.

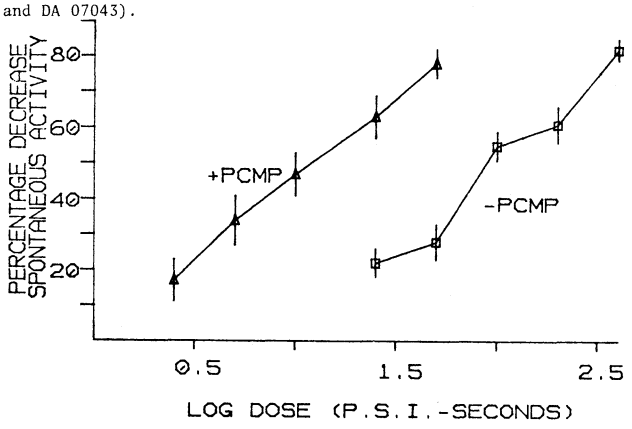
Preincubation of brain homogenate at 37° for 20 min prior to incubation with [^{3}H]diazepam at 0° increased binding activity by 40-50%. Preincubation of membrane preparation at 37°, however, did not result with similar increase in binding activity. Furthermore 6-heptyldithioguanine, but not 6-benzylthioguanine, was less effective in inhibiting [^{3}H]diazepam binding after preincubating with the homogenate at 37°.

Similar to previous observations with 6-methylthioguanine, both 6-benzylthioguanine and 6-heptyldithioguanine were less effective in inhibiting [^{3}H]diazepam binding in developing (6-day old) rat brain than in adult, using either brain homogenates or membrane preparations.

Supported by the Medical Research Council of Canada.

102.6 SIMILARITY OF ELECTROPHYSIOLOGIC EFFECTS OF PHENCYCLIDINE AND DOPAMINE IN CAUDATE. S.W. Johnson*, P.E. Haroldsen*, B.J. Hoffer*, and R. Freedman*. (SPON: M.L. Reite). Departments of Pharmacology and Psychiatry, University of Colorado Medical Center, Denver, Colorado 80262.

The effects of dopamine, phencyclidine, and stereoisomers of the 3-methyl derivative of phencyclidine (+PCMP, -PCMP) were studied in the caudate of urethane-anesthetized rats. Each drug was dissolved in saline and pressure-ejected from multi-barrel micropipettes. Spontaneous and cortically-evoked single unit discharge of caudate neurons were recorded extracellularly. In 73 neurons studied, inhibition of spontaneous activity was produced by phencyclidine in 81% of neurons, while dopamine inhibited 77%. An increase in spontaneous activity was produced by phencyclidine in 9% and by dopamine in 13%. Both phencyclidine and dopamine exerted a modulatory effect on neuronal discharge by producing inhibition of spontaneous activity at "doses" which did not affect activity evoked by stimulation of cerebral cortex. Depressant effects of phencyclidine and dopamine could be reversibly blocked by concurrent pressure-ejection of fluphenazine (7/12 neurons). Furthermore, +PCMP, the behaviorally more effective isomer, was found to be 8 times more potent than -PCMP for the reduction of caudate neuronal spontaneous activity (see figure below). These results suggest that phencyclidine has pharmacologically specific effects in the caudate, which are similar to those of dopamine. (Supported by DA 02429, NS 09199, and DA 07043).



102.7 TIME DEPENDENT ALTERATIONS OF LEVODOPA TREATMENT ON PRE-SYNAPTIC DOPAMINE NEURONAL FUNCTION AND POST-SYNAPTIC DOPAMINE RECEPTORS. K.D. Wilner, I.J. Butler, W.E. Seifert* and Y.C. Clement-Cormier. Depts. of Pharmacology, Neurobiology and Biochemistry, University of Texas Medical School, Houston, Texas 77025.

In this study we examined the effect of acute and chronic levodopa treatment on D_1 and D_2 dopamine receptors and on tyrosine hydroxylase activity in the rat striatum. A combination of levodopa (1000 mg/kg) and carbidopa (100 mg/kg) was administered to rats orally for 1 (acute dose) to 28 days. At selected time intervals, animals were sacrificed and the following measurements were made: (1) dopamine-sensitive adenylate cyclase (D_1 receptor activity); (2) (^{3}H) spiroperidol binding, (D_2 receptor binding site); and (3) tyrosine hydroxylase. The data reveal biphasic changes in both dopamine-sensitive adenylate cyclase activity and (^{3}H)spiroperidol binding. Following acute administration of levodopa, a 30% decrease in the maximal level of dopamine stimulation of adenylate cyclase was detected in the drug treated group with no change in the EC50 for dopamine. Acute levodopa treatment also produced a 60% increase in the dissociation constant (Kd) for (^{3}H)spiroperidol binding with no change in the Bmax. Conversely, after chronic drug treatment for $\geq$ 21 days, the EC50 values for dopamine stimulation of adenylate cyclase increased by two fold with no change in the maximal level of activity and the Bmax for (^{3}H)spiroperidol binding decreased by 30% with no change in the Kd value. Whereas acute treatment did not significantly change tyrosine hydroxylase activity, a two fold increase in the Vmax was detected in the drug treated group after 21 days. In summary, these results demonstrate that down regulation of the dopamine receptor can occur following exposure to levodopa. However, it appears from our data that there is a differential response of D_1 and D_2 dopamine receptors following agonist treatment and that this response may be influenced by pre-synaptic mechanisms as the time period for drug treatment increases.

102.8 GTP DEPENDENT ACTIVATION OF RAT BRAIN ADENYLATE CYCLASE IS ENHANCED BY CHRONIC ANTIDEPRESSANT TREATMENT. Marcia A. Wheeler*, David B. Menkes, and Mark M. Rasenick (SPON: William H. Miller). Depts. of Pharmacology and Neurology, Yale Univ. School of Medicine, New Haven, CT 06510.

Clinical response to antidepressant therapy(AD) does not generally develop until one or more weeks following treatment. Studies of the biochemical and cellular effects of AD must therefore focus on changes elicited by chronic treatment. Altered neurotransmitter sensitivity and receptor binding characteristics have been detected in a number of systems, and have been proposed as an underlying mechanism of AD action. Adenylate cyclase (AC) may transduce the effect of a number of neurotransmitters, the sequence of such transduction within the AC cascade being: Transmitter receptor → GTP binding regulatory subunit(G-unit) → Catalytic moiety. As chronic AD treatment may affect membrane properties which alter AC through the G-unit (Nature:294, 560, 1981), it was thought that chronic AD treatment might also affect AC through the G-unit.

Rats received either chronic or acute electroconvulsive treatment or antidepressant drugs. Treatment of rats with daily injections of amitriptyline(AMI), desiprimine(DMI), or imiprimine(IMI) for 15 days resulted in increased activation of AC, from frontal cerebral cortex or hypothalamus, assayed in the presence of guanylylimidodiphosphate [Gpp(NH)p] or NaF. A similar effect was seen in membranes prepared from frontal cerebral cortex of rats treated for 14 days with trans-sinusoidal current (ECT). Acute treatment with AMI, DMI, IMI, or ECT did not alter AC activity, nor did in-vitro addition of the above drugs.

Chronic AD effects on AC were similar to those of in vitro addition of colchicine(CL) or vinblastine(VB) (Nature:294:560, 1981). AC activation by Mn^{2+}, which is reflective of activity independent of the G-unit, was not altered by CL, VB or chronic AD treatment, despite the twofold increase in GPP(NH)p or NaF activation caused by these agents. The effects of chronic AD treatment plus CL or VB are not additive.

It is possible that chronic AD treatment serves to augment AC G-unit/catalytic moeity association. Although these studies demonstrated enhanced AC activation, chronic AD treatment could result in either increased or decreased AC activity, as AC inhibition through α_2 receptors appears to require a G-unit (Mol. Pharmacol., 21:17, 1982). At least some of the effects of chronic AD treatment might thus be expressed distal to the receptor through the AC G-unit.

102.9 AGING AND BRAIN RECEPTOR CHANGES DURING CHRONIC FLUPHENAZINE TREATMENT. C.H. Misra, H. Shelat*, V. Patel*, I. Irabor* & R.C. Smith . Biological Psychiatry, Texas Research Institute for Mental Sciences, Houston, Texas, 77030.

Supersensitivity of dopaminergic and other receptors recurrs after chronic administration of neuroleptic drugs to the rat; these receptor changes may be related to the underlying pathophysiology of Tardive Dyskinesia. Tardive Dyskinesia is much more prevalent in geriatric patients receiving chronic treatment with neuroleptics. We have previously shown that old rats (25 months) develop a greater increase in the B_{max} of β-adrenergic than 7 month old rats, 9 days after termination of chronic fluphenazine (FLU), but have a similar increase in specific binding of spiperone (Misra et al., Eur. J. Pharm. 76, 317-324, 1981). We now report differential effects of age on receptor changes in the rat brain during the period of chronic FLU administration. Old (25 month) rats showed a quantitatively greater (203%) increase in the K_D of spiperone binding and on earlier onset (2 weeks) of increase in the K_D of spiperone bonding than young rats. The young (7 month) adult rats showed a smaller (87%) increase in the K_D after 6 weeks of FLU but not at two weeks of FLU. However, the 7 month old rats showed a significant increase of 25% in the B_{max} of spiperone binding after 6 weeks of FLU, whereas the old rats showed no increase in the B_{max} of spiperone binding. The 7 month rats showed a 13% increase in B_{max} of DHA binding after 2 weeks FLU but a return to baseline levels by 6 weeks, whereas the 25 month old rats showed no change in the B_{max} of DHA binding at 2 weeks of FLU, but a significant 14% increase after 6 weeks of FLU. Studies of receptor binding changes in 4 age groups terminated from chronic FLU also currently in progress and will be reported.

102.10 KINETIC STUDIES OF ALPHA-ADRENERGIC RECEPTOR-MEDIATED RESPONSES IN THE RABBIT AORTA. R. N. Cory*, R. Osman* and S. Maayani. Dept. of Pharmacology, Mt. Sinai School of Medicine, N.Y., N.Y. 10029.

Pharmacological responses are usually characterized by thermodynamic parameters. This method of characterization disregards the time dependence of the response elicited by the drug. The analysis of the time dependence of pharmacological responses necessitates the formulation and testing of specific kinetic models; thus, the kinetic model that best fits the experimental data also provides a mechanistic description for receptor mediated events of the pharmacological response.

We investigated the relation of the response to the agonist concentration and tested kinetic models for the response. These models are based on events related to drug-receptor interactions and temporal changes in the drug-receptor complex that may be linked to the generation of the response. The alpha-adrenergic agonist phenylephrine (acting exclusively on the $alpha_1$ receptors in this system, ED_{50} = 0.15 uM) elicits three types of dose-dependent response profiles: 1) at low concentrations (0.03 - 0.1 uM) there is a fast phase that is completed within 1 minute and accounts for 30-40% of the response amplitude; the fast phase leads into a slow response that takes 20-30 minutes to stabilize, 2) at mid-range (0.1-3 uM) the fast phase comprises 30-60% of the response and merges with the slow phase which is over within 10 min, and 3) at high concentrations (3-100 uM) the fast phase rises rapidly within 1 minute and accounts for 70-90% of the response amplitude; following the fast phase there is a plateau that leads into the slow phase which stabilizes within a few minutes. The response decrement after drug wash out is also biphasic with an initial fast phase followed by a slow phase. A similar response pattern is observed with histamine, but, other adrenergic agonists which do not elicit a maximal response (partial agonists) exhibit a different kinetic profile.

In order to interpret the above observations we constructed kinetic models of the response. In these models the magnitude of the response was assumed to be related to the amount of drug-receptor complex. A simple bimolecular mechanism ($D + R \leftrightarrows DR$) cannot account for the behavior of this system. A more intricate model with sequential steps, involving D-R transformations ($D + R \leftrightarrows D\text{-}R_1 \leftrightarrows D\text{-}R_2$), or post-synaptic events (e.g. mobilization of Ca^{++} pools) accomodates the biphasic response patterns observed. The application of the kinetic model to this system allows us to construct specific tests to identify the various stages between the drug-receptor interaction and the response generation. (Supported by an Adv. Predoct. PMA Award to R.N.C. and USPHS grant DA-01875)

102.11 DISSOCIATION OF DECREASED cAMP RESPONSE AND BETA-ADRENERGIC RECEPTOR DOWNREGULATION IN THE RAT PINEAL GLAND FOLLOWING ADMINISTRATION OF DESMETHYLIMIPRAMINE. J. A. Moyer, E. A. Muth* and E. B. Sigg*, Department of Pharmacology, Wyeth Laboratories, Inc., P.O. Box 8299, Philadelphia, PA 19101.

Previous experiments have shown that repeated desmethylimipramine (DMI) treatment reduces the ability of isoproterenol (ISO) administered either in vivo or in vitro to elevate concentrations of 3', 5' - adenosine monophosphate (cAMP) in rat pineal glands (Life Sciences 24, 2237, 1979; Molecular Pharmacology 19, 187, 1981). This effect was believed to be due to a decrease in β-adrenergic receptor number and a reduction in norepinephrine-stimulated adenylate cyclase activity. Current studies examine the time course of induction of noradrenergic subsensitivity in male Sprague-Dawley rats exposed to constant light following DMI treatment (10 mg/kg, ip, twice a day for periods of 1-5 days) by determining ISO-stimulated (2 μmoles/kg, ip) cAMP production and β-adrenergic receptor number using ^{3}H-dihydroalprenolol as a ligand.

A maximal decrease (78%) in ISO-stimulated cAMP concentrations was noted following only 3 injections of DMI (2 days treatment), while the greatest decrease (20%) in ^{3}H-dihydroalprenolol binding was not observed until 10 injections (5 days treatment). To examine this further, additional rats were injected with a single dose of DMI (10 mg/kg, ip) and cAMP responsiveness and ^{3}H-dihydroalprenolol binding were measured at several times over a 48-hour period. A maximal decrease (65%) in the cAMP response to an ISO challenge was observed at the 24-hour period, with a return to normal values by 48 hours; however, there was no significant effect on β-adrenergic receptor number during these time periods. In these studies, single and repeated treatments with DMI resulted in changes in cAMP responsiveness which were temporally and quantitatively dissociated from the alteration in β-adrenergic receptor number. This suggests that DMI may affect cellular mechanisms independent of the β-adrenergic receptor.

102.12 THE INFLUENCE OF ACTH AND IMIPRAMINE ON β-RECEPTOR BINDING AND CATECHOLAMINE-STIMULATED cAMP ACCUMULATION IN RAT BRAIN FRONTAL CORTEX AFTER LESION OF THE DORSAL BUNDLE. R. Duman* and S.J. Enna (SPON: S. Strada). Depts. of Pharmacology and of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77025.

It has been reported that ACTH reduces the lag time necessary for observing a significant decline in β-adrenergic receptor binding and function in rat brain frontal cortex that results from chronic administration of imipramine. To determine the importance of presynaptic activity in this response, noradrenergic innervation was diminished by destroying the rat brain dorsal bundle with 6-hydroxydopamine. Twelve days after surgery, imipramine (10 mg/kg, i.p., once daily), ACTH (Acthar gel, 50 i.u./kg, s.c., once daily) or the combined treatment was initiated and continued for 4 days. Sixteen hours after the last injection the animals were killed and β-receptor binding and NE-stimulated cAMP accumulation analyzed in frontal cortex. β-Receptor number and affinity were determined by measuring ^{3}H-dihydroalprenolol binding and cAMP was analyzed by radioimmunoassay.

Whereas the ACTH-imipramine combination decreased β-receptor binding and ACTH by itself had no effect in unoperated controls, with lesioned animals the drug combination and peptide alone induced an increase in receptor number. This increase was greater than that found in untreated lesioned animals. In contrast, catecholamine-stimulated cAMP accumulation was increased in both the treated and control lesioned animals with respect to unlesioned controls. These findings suggest that noradrenergic terminals are necessary for mediating the ACTH + imipramine-induced decline in β-receptor number. Moreover, it would appear that ACTH modifies the responsivity of the cyclic nucleotide system by influencing noradrenergic activity presynaptically. However, ACTH may also have a postsynaptic site of action since a more rapid increase in β-receptor number was noted in lesioned animals following treatment with this peptide. Thus ACTH, or adrenocroticoids, may serve to sensitize neuronal membranes such that adaptive responses to receptor occupancy can occur more readily.

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102.13 ROLE of β-ADRENERGIC RECEPTORS (β-AR) IN THE ANTIDEPRESSANT ACTIVITY OF ALPRAZOLAM. V.H.Sethy and D.H.Hodges, Jr.*, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Antidepressants have latent onset of therapeutic effects. This is consistent with the preclinical observation that chronic administration of antidepressant drugs decreases norepinephrine-stimulated cyclic adenosine-3',5' monophosphate formation and density of β-AR in the cerebral cortex of rats. Similar effects are not seen after acute injection of these drugs. If β-AR play an important role in the mediation of therapeutic effects of antidepressants, then it is important to study the importance of this receptor system in antidepressant activity of alprazolam, a clinically effective triazolobenzodiazepine. Female rats were implanted with polyethylene cannula in the jugular vein. Desipramine, alprazolam, or diazepam (each 10 mg/kg/day) were chronically infused through the venous cannula. Reserpine was either injected intraperitoneally (1 mg/kg/3rd day) or infused (0.1 mg/kg/day) chronically. Reserpine significantly ($p<0.001$) increased the density of β-AR in the cerebral cortex. Desipramine significantly ($p<0.001$) reduced β-AR as reported previously (Sethy and Harris, Drug Devel. Res. 1982) and also blocked reserpine-induced increases in β-AR. Chronic treatment with alprazolam and diazepam had no significant effect on the β-AR. However, alprazolam, when administered along with reserpine, significantly ($p<0.015$) blocked reserpine-induced increases in β-AR. Under similar conditions, diazepam had no significant effect on reserpine-induced elevation in β-AR. These results suggest that alprazolam may mediate its antidepressant activity through β-AR.

102.14 THE SPECIFIC BINDING OF [^{3}H]NITRENDIPINE TO CALCIUM BINDING SITES IN THE RAT CEREBRAL CORTEX. E. Itoga*, W.R. Roeske*, F.J. Ehlert*, S.Kito and H.I. Yamamura (Spon: W. Barber). Dept. of Pharmacology, Univ. of Arizona, Tucson, AZ 85724 & 3rd Dept. of Internal Medicine, Hiroshima, Japan 734.

We have recently demonstrated that [^{3}H]nitrendipine interacts with receptors for calcium antagonists in the rat cerebral cortex and heart (Biochem. Biophys. Res. Comm. 104:937, 1982). In this study, we present further data on the characterization, drug and ionic specificity of specific [^{3}H]nitrendipine binding in the rat cerebral cortex. Standard cerebral cortical membranes were prepared in 50 mM Tris-Hepes buffer, pH 7.4. Radiolabeled nitrendipine was incubated for 60 minutes at 25°C. Specific binding was defined as the difference in counts bound in the presence and absence of 1 uM nifedipine. Under these conditions, the dissociation constant from Scatchard plots was approximately 200 pM and the maximal number of binding sites was approximately 180 fmol/mg protein. Kinetic studies were also performed and a dissociation constant of about 40 pM was obtained from equilibrium association and dissociation studies. Non-equilibrium dissociation studies were also done to determine whether the dissociaion rates were similar under pre-equilibrium and equilibrium conditions. The 50% dissociation times were significantly different at pre-equilibrium and equilibrium conditions thus providing evidence for receptor isomerization. A number of calcium antagonists were examined for their ability to inhibit [^{3}H]nitrendipine binding in cortical membranes. The order of potency was nitrendipine>, niboldipine>, nifedipine>, nimodipine>, verapamil>, methoxyverapamil>, diltiazem. Interestingly at 0.25 nM [^{3}H]nitrendipine, the maximal inhibition observed by verapamil was about 50%, thus providing evidence for negative heterotropic cooperativity. We examined a number of ions for their ability to inhibit [^{3}H]nitrendipine binding. Among the ions examined, Cd^{++}, Ni^{++}, Mg^{++}, Mn^{++} and Ca^{++} were effective inhibitors of [^{3}H]nitrendipine binding while Na^+ and K^+ were ineffective ions. We conclude that [^{3}H]nitrendipine binds specifically to cerebral cortical sites with high affinity; the drug and ionic specificity support the concept that these binding sites are integral parts of the calcium channel and that this newly developed radioligand will prove useful in determining the molecular mechanisms of the calcium channel. Supported by NIMH grants and a RSDA to W.R.R. and H.I.Y.

102.15 POSSIBLE INVOLVEMENT OF ß-CARBOLINES IN THE STRESS-INDUCED REDUCTION OF CENTRAL GABA RECEPTORS. G.Biggio, A. Concas*, M.Salis*, M.Serra* and M.G.Corda*. Institute of Biology, Chair of Pharmacology, University of Cagliari, Italy.

Brain of unstressed rats(rats accustomed to the handling preceding sacrifice by guillotine)has a higher total number of ^{3}H-GABA binding sites than brain of stressed rats(naive animals)(Biggio et al.,Brain Res., 229,363,1981). ^{3}H-GABA binding was studied in different brain areas of stressed and unstressed rats after in vitro addition of diazepam and benzodiazepine antagonists Ethyl-ß-carboline-3-carboxylate(ß-CCE) 6,7Dimethoxy-4-Ethylcarboline-3-carboxylic-acid-methylester(DMCM) and Ro15-1788.Diazepam($5x10^{-6}$M) increased (by 30%) total number of ^{3}H-GABA binding in brain tissue from stressed rats but failed to modify ^{3}H-GABA binding in tissue from unstressed animals.ß-CCE,DMCM and Ro15-1788 ($5x10^{-6}$M) decreased (by 40%) the total number of GABA binding sites in brain tissue from unstressed rats but failed to further decrease ^{3}H-GABA binding in tissue from stressed animals. Changes in affinities of GABA binding sites for ^{3}H-GABA were inversely related to changes in the number of receptors. Stimulant effect of diazepam on GABA binding was eliminated after several washings of membranes with buffer solution while the inhibitory effect of ß-CCE persisted. Similarly repeated washing of brain membranes from stressed rats failed to increase GABA binding. The findings indicate: a)Diazepam antagonizes stress-induced decrease in GABA binding but fails to modify GABA receptors of unstressed animals. b)Benzodiazepine antagonists decrease GABA binding in unstressed animals, but not in stressed ones. c) Decrease in GABA binding induced by stress or by benzodiazepine antagonists is not reversed by washing. It is suggested that stress decreases GABAergic transmission by releasing endogenous ß-carbolines.

103.1 ONSAGER RECIPROCAL RELATIONS INVOLVING THE ACTIVE TRANSLOCATION OF SODIUM AND POTASSIUM IONS ACROSS THE NEURONAL MEMBRANE. G. M. Schoepfle, J. T. Tarvin* and R. M. Martin. Neurosciences Program, Univ. of Alabama Med. Ctr., Birmingham, AL 35294.

A recent theoretical analysis of tetanic hyperpolarization involves the coupling of an active transport current term to the Frankenhaeuser-Huxley excitation equations for the Xenopus node action potential (Schoepfle, G. M., J. T. Tarvin & R. M. Martin. Biophys. J. 33:125, 1981). Active sodium and potassium current density terms are $(\nu_{Na}/n)g_p T\Delta S$ and $-(\nu_K/n)g_p T\Delta S$ where g_p is a pump conductance and the ΔS term represents the classically derived total entropy change involved in the reversible splitting of one mole of ATP via the electrogenic pump reaction

$$\nu_{Na}Na_i + \nu_K K_o + nATP + nH_2O \rightarrow \nu_{Na}Na_o + \nu_K K_i + nADP + nP_i$$

If now the active transport translocation terms for sodium and potassium are expressed as molar fluxes the total system of linked fluxes, including the rate of ATP hydrolysis, can be represented as the matrix

$$\begin{pmatrix} M_{Na} \\ M_K \\ M_{ATP} \end{pmatrix} = \begin{pmatrix} -(\nu_{Na}/n)^2 B & [(\nu_{Na}\nu_K)/n^2]B & -(\nu_{Na}/n)B \\ [(\nu_{Na}\nu_K)/n^2]B & -(\nu_K/n)^2 B & (\nu_K/n)B \\ -(\nu_{Na}/n)B & (\nu_K/n)B & -B \end{pmatrix} \begin{pmatrix} E_{Na} \\ E_K \\ E_o \end{pmatrix}$$

$$+ \begin{pmatrix} [\nu_{Na}(\nu_{Na} - \nu_K)/n^2]B & 0 & 0 \\ 0 & -[\nu_K(\nu_{Na} - \nu_K)/n^2]B & 0 \\ 0 & 0 & [(\nu_{Na} - \nu_K)/n]B \end{pmatrix} \begin{pmatrix} V_m \\ V_m \\ V_m \end{pmatrix}$$

where all M terms are given in terms of moles per square cm per s, and B is defined as (g_p/F). The emf term E_o becomes

$$E_o = g_p(RT/F)\ln[(ADP)(P_i)/(ATP)(H_2O)] + g_p(\Delta H/F) + g_p(T/F)[S^o_{ATP} + S^o_{H_2O} - S^o_{ADP} - S^o_{P_i}]$$

where each S term is a standard entropy, and ΔH is the enthalpy change of the system. While Rapoport (Rapoport, S., Biophys. J. 20:246, 1970) in a more generalized treatment involving irreversible thermodynamics did derive identical expressions for the ΔS term, and whereas Rapoport did indicate the identity of two sets of cross coefficients for current densities it is established here that a derivation involving purely classical thermodynamics yields a complete set of symmetrically identical cross coefficients for all linked fluxes. (NIH Support).

103.2 DIFFUSION COEFFICIENT MEASUREMENTS OF NEUROTRANSMITTER-RELATED COMPOUNDS. Greg. A. Gerhardt* and Ralph N. Adams. Department of Chemistry, University of Kansas, Lawrence, KS 66045.

In recent years our laboratory and others have been interested in diffusion processes which occur in the complex matrix of the brain. In particular, we have been interested in the observed or apparent diffusion coefficients of molecules in the brain as compared to their solution values. The quest for precise and accurate solution diffusion coefficients is, in itself, not a trivial matter. Techniques employing electrochemistry, capillary diffusion and diffusion from a point source are often limited to the study of certain compounds and are far from routine. We have developed a technique which involves measuring solution diffusion coefficients based on the dispersion of a sample in a flow stream. The method is based entirely on analytical expressions of dispersion in a flow stream derived by Vanderslice (Talanta 28:11, 1981). The result is a fast, reliable method for the precise and accurate determination of solution diffusion coefficients of a wide variety of compounds. A major advantage of this technique is that it allows for the use of a multitude of detectors, which greatly increases the number of compounds which can be studied.

We have applied this method to the study of the solution diffusion coefficients of many of the biogenic amine neurotransmitters and their metabolites. Other putative neurotransmitters such as leu-enkephalin, and drug molecules have been explored. A list of a few solution D values in 0.1 M phosphate buffer, pH 7.4, at 25° C is as follows:

Compound	D X 10^5 cm^2/sec	n
Dopamine (DA)	$.60_5 \pm 0.025$	19
Norepinephrine (NE)	$.55_6 \pm 0.026$	7
Phencyclidine (PCP)	$.47_9 \pm 0.027$	9
Ketamine	$.56_6 \pm 0.026$	9
Leu-enkephalin	$.41_8 \pm 0.014$	6
d-Amphetamine	$.61_1 \pm 0.012$	8

Additional peptides and drug molecules have been studied. Comparisons between solution and observed diffusion coefficients in the brain have been explored and show promise for aiding in the understanding of the complex microenvironment of the brain.

103.3 ELECTROPHYSICAL EFFECTS OF GENERAL ANESTHETIC GASES. S.R.Hameroff, R.C. Watt,* B.Lemay* and K. Mylrea.* Depts. of Anesthesiology and Elect. Engineering, U. of Ariz. Health Sci. Ctr., Tucson, AZ 85724

Anesthetic gas molecules have electron cloud dipole characteristics and electronegativity which promote low-energy Van der Waal's bonding (Wulf, R.J., Featherstone, R.M., Anesthesiology, 47:532, 1957). These properties can inhibit electron mobility and excitation such as luminescence from firefly luciferase and various bacteria (Halsey, M.J., Smith, E.B., Nature, 227:1363, 1970). Since protein conformational regulation may be determined by excited electron dipole oscillations (Frohlich, H., Proc. Nat. Acad. Sci.,72:4211, 1975) and since anesthetics act by inhibiting membrane protein conformational responsiveness, we have studied direct effects of anesthetics on electron mobility in a non-biological system of corona discharge.

An E-field generator delivers exponentially decaying 20 kV, 10 kHz sinusoidal bursts at 60 Hz repetition rate to an excitation chamber and dielectric which consists of concentric cylindrical electrodes. The field strength within the 1 cm chamber compares to neuronal intra-membrane field strengths (~100 mV/100 Å = 10^5 V/cm). The voltage induces a capacitively coupled current through the excitation chamber. When a non-anesthetic "carrier" gas (oxygen or air) is in the chamber, corona discharge occurs which results in photon emission and current spikes of nanosecond duration which may be superimposed on the generator output waveform. The generator waveform may be filtered out,leaving the discharge spikes which are electronically counted.

If anesthetic gases are added to the "carrier" gas the corona discharge is rapidly and reversibly quenched in logarithmic proportion to anesthetic concentration. The anesthetics inhibit propagation of electron avalanches via electron attachment, dielectric alteration, or excitation energy absorption. Halothane, enflurane, and isoflurane are much more potent quenchers than nitrous oxide, and corona inhibition in our system is proportional to anesthetic clinical potency. Preliminary data show the phenomenon to be independent of gas flow, humidity and temperature. Our studies suggest that this basic electrophysical characteristic is common to all anesthetics. Non anesthetic gases (helium,carbon dioxide at low concentrations) do not inhibit corona discharge. Excited electron states spatially arrayed in membrane or cytoplasmic protein matrices may be an important information processing mode (Hameroff, S.R., Watt, R.C., Naval Res. Mem. Rep.. 4662: 292, 1981). Anesthesia may thus occur due to inhibition of electronic excitation and/or mobility within hydrophobic protein regions which are responsible for membrane or cytoplasmic protein function.

103.4 AUTOMATED DETECTION OF MEMBRANE POTENTIAL CHANGES USING FLUORESCENT DYES IN SYNAPTOSOMES AND MICROSACS. M.R. McBride*, J.A. Monti, and S.T. Christian. Neurosciences Program, Univ. of Alabama in Birmingham, Univ. Station, Birmingham, AL 35294.

The monitoring of the membrane potential of small vesicles has been proven essential for delineation of the effects many membrane acting agents exert on localized neuronal regions. The quantification of synaptic membrane potential changes can give information about the mechanism by which these agents act. Pre-synaptic release sites have been extensively modeled by the synaptosome and in the last few years the microsac has been proposed as a possible post-synaptic model.

The carbocyanines, oxanols and other ionic dyes respond to changes in transmembrane potentials by changes in fluorescence intensity (ΔF). Agents which affect the membrane potential cause a concomitant change in fluorescence. While the technique is appealing because of its simplicity there are several limitations. One of these limitations is the difficulty in quantitatively correlating the ΔF with shifts in membrane potential. Background fluorescent noise can cause a second problem by degrading the S/N ratio. Pharmacological agents that increase or decrease the rate of potential change, not the final equilibrium potential, must be detected by rate and not steady state measurements.

A technique has been developed in this laboratory utilizing a SLM 4800 spectroflurometer system that provides an easy, rapid and accurate means for monitoring changes in potential in synaptosomes and microsacs. Computer control allows rapid and precise intensity measurements with long term data storage and analysis techniques providing an enhanced S/N ratio; increasing the accuracy for measurement of small signal changes. Calibration of ΔF and membrane potential changes was accomplished with K^+ depolarizing and Cl^- hyperpolarizing conditions. Experiments performed with increasing K^+_o allowed the generation of a standard curve usable for depolarizing agents and by increasing Cl^-_o a hyperpolarizing standard curve was generated. In each experiment tetrodotoxin was used to block Na^+ flux and all other ion concentrations were held constant. Batrachotoxin (BTX) was used between 1 μM and 10 μM to verify the viability of measuring membrane depolarization rate. The rate for 10 μM BTX was significantly greater than 1 μM BTX while the final equilibrium depolarized fluorescence was the same.

This system allows the study of agents that modify ion flux activity at the pre-synaptic and possible post-synaptic sites. Screening and analysis of agents which effect Na^+, K^+, Cl^- fluxes are currently in progress. Supported by NIH HD11893.

103.5 EFFECT OF ETHANOL ON THE SLOW TUMBLING OF MEMBRANE BOUND PROTEIN MEASURED BY SATURATION TRANSFER EPR. (C.E. Swenberg,* M.J. McCreery,* and H. Pant (Spon. L.G. Cockerham). Radiation Sciences Department, AFRRI, Bethesda, MD 20814 and Lab. Preclinical Studies, NIAAA, Rockville, MD 20852.

Recent observations have suggested that ethanol alters the physiologic state of biologic membranes by increasing membrane fluidity (J. H. Chin and D. B. Goldstein, Science 196: 684, 1976). These observations seem to correlate well with states of intoxication in rodents acutely treated with ethanol (R. C. Lyon, et al. J. Pharmacol. Exper. Therap. 218: 669, 1981). It has further been suggested that the biological response in membranes to chronic ethanol administration is an increase in the relative amounts of cholesterol constituting the lipid bilayer (D. A. Johnson et al., Molec. Pharm. 15; 739, 1979). These data have been used to explain the development of tolerance to ethanol and also the withdrawal syndrome upon subsequent removal of this drug. However, the order parameter S of the lipid spin labels used to infer these fluidity perturbations correspond to changes of less than 1% in S even at very high ethanol concentrations. We report here that the slow rate of tumbling exhibited by membrane proteins is increased by an order of magnitude by ethanol concentrations between 1% and 5% in rat synaptosomal plasma membranes (SPM). Rats were killed by cervical dislocation, their brains quickly removed and homogenized in 10 volumes of ice cold sucrose (0.32 M sucrose, 4mM Tris-HCl, pH=7.4). Synaptosomal plasma membranes were made by the technique of Gray and Whittaker (J. Anal. (Lond). 96:78, 1962). For most experiments up to six rat brains were pooled in order to enhance signal to noise. Membrane proteins were labelled covalently and non-specifically with 4-maleimido -2, 2, 6, 6 -tetramethylpiperidinooxyl by dissolving the probe in buffer and incubating at 4°C with membranes for 12 hours. Free probe was removed by repeated centrifugation. EPR spectra were recorded using a Varian E-109 spectrometer operating at X-band in the absorption mode. The spectrometer was interfaced with a Nicolet 1180 computer for analysis of data. In phase 100 KHz spectra consisted of both an immobilized and a very mobile component. Correct phasing of the spectrum was determined under low power (2mW) conditions and the second harmonic (50KHz) out-of-phase absorption spectra were measured at 32 mW with 5 gauss modulation amplitude; signal averaging (up to 500 scans) was found to be essential for high signal to noise ratio spectra. The addition of ethanol (1% and 5% by vol.) significantly altered the L-region of the saturation transfer spectra. Our results indicate that relative to control SPMs, ethanol depressed the L" peak while slightly enhancing the L peak. The very mobile component was quenched by the addition of either $NiCl_2$ or sodium ascorbate. Membranes exposed to ethanol showed a more significant reduction in the intensity of the EPR signal relative to controls. Our results suggest that in vitro exposure of the SPMs to ethanol produces a more fluid environment for membrane proteins and a greater exposure to the aqueous media.

103.6 MEASUREMENT OF SHIFT IN SUM OF PHASE BOUNDARY POTENTIAL FROM PERMEABILITY DATA. A. E. Lacerda* and T. L. Schwartz*. (SPON: J. B. Tuttle). Physiology Section, Biological Sciences Group, University of Connecticut, Storrs, Ct. 06268.

Schwartz and Kado (Biophys. J. 18:323, 1977) introduced a new approach to ionic permeability based on diffusion theory but with no need for the classical assumptions of constant field, homogeneous membrane and equal phase boundary potentials. They defined a general permeability, A/Q', free of these constraints. A/Q' can be evaluated directly from current-voltage (I-V) data and is a function of both the transmembrane potential and the concentration of the permeant ion. A/Q' becomes concentration independent when the transmembrane potential is equal to the sum of the phase boundary potentials. This sum can therefore be determined.

I-V data taken from a potassium selective, slow (Marty and Ascher, Nature 274:494, 1978) cholinergic channel found in the medial cells of Aplysia californica (Kehoe, J. Physiol. 225:85, 1972a,b) are fitted by a nonlinear least squares procedure to an equation of the form: $I = B \exp(mE) + I_\infty$ where I and E are, respectively, the voltage clamped current due to the application of carbachol and the transmembrane potential. B, m and I_∞ are constants obtained from the fitting procedure. This equation has been found to give a good description of the I-V relation for this channel over a wide range of conditions at times shorter than the mean channel lifetime. The currents obtained from the above expression are used to calculate A/Q':

$$A/Q' = I / FRT\, a(sl) \left(e^{F/RT(E-E_o)} - 1 \right)$$

F, R and T have their usual meaning, a(sl) is the activity of potassium ion in the external bath and Eo is the reversal potential for potassium.

When A/Q' for different external potassium concentrations is plotted against transmembrane potential the A/Q' curves intersect at one point, within experimental error. The potential at the common point of intersection, γ, is the sum of the phase boundary potentials (Schwartz and Kado, 1977). A fivefold increase in the concentration of calcium in the external solution is expected to cause the external phase boundary potential to move in a depolarizing direction in an amount that would depend on a model of the surface charge producing mechanism. Assuming the internal phase boundary potential remains unaltered by the increase in external calcium, the change in the sum of the phase boundary potentials will reflect shifts in the potential of the external phase boundary. We have measured this shift and find it to be +38 millivolts in the depolarizing direction. This provides experimental support for the hypothesis that γ is the sum of the phase boundary potentials and lends support to the generalized approach to permeability.

103.7 ELECTROTONIC LOCALIZATION OF HIPPOCAMPAL MOSSY FIBER SYNAPSES. Thomas H. Brown and Daniel Johnston. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010 and Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

Several features of the hippocampal mossy fiber (mf) synaptic system motivate its attractiveness for studies of the microphysiology and biophysics of synaptic transmission in the mammalian cortex (See Brown, et al, Brain Res. 177:194, 1979; Brown, et al, J. Neurophysiol. 46:812, 1981; Johnston, Cell. Mol. Neurobiol. 1:41, 1981). Like other hippocampal synapses, these also display interesting forms of use-dependent plasticity, including long-term synaptic potentiation (LTP), which can last for hours and perhaps even days or weeks. We were interested in investigating the mf synaptic currents using the recently developed single-electrode voltage clamp (SEC) (Johnston, ibid; Johnston, et al, Nature 286:391, 1980; Johnston & Brown, Science 212:294, 1981). Our previous modeling studies (Johnston & Brown, Neurosci. Abstr. 7:517, 1981; in preparation) had shown that the SEC will provide accurate synaptic current measurements for local conductance changes within the frequency band of interest (0-100 Hz). However, these same studies demonstrated that the measured current waveforms become considerably distorted and attenuated when the conductance changes are located at an electrotonic distance X greater than about 0.1 from the recording site. We therefore wanted to know the electrotonic distance from the soma to the mf synaptic region.

Electrophysiological and anatomical data were combined to give three estimates of the electrotonic distance from the soma to the mf synapses. The three methods, to be discussed, gave similar results. The following table summarizes the anatomical and electrotonic distances from the soma to portions of the mf synaptic region (n = 20). The electrotonic distance from the soma to the most distal mf synapses averaged only 0.06, while the electrotonic distance from the soma to the most proximal mf synapses averaged only 0.01. We condlude that the mf synapses are electrotonically sufficiently close to the soma to permit reasonably accurate voltage-clamp measurements of the synaptic currents. Such measurement may provide useful insights into the mechanisms underlying LTP. (Supported by McKnight Foundation Scholars and Development Awards, & NIH Grants NS-16576, 11535 and 15772.)

Summary	Anatomical Distance (μm)			Electrotonic Distance (X)		
Statistic	Proximal	Midpoint	Distal	Proximal	Midpoint	Distal
mean	18	82	146	.01	.03	.06
median	5	75	145	.00	.02	.06
range	0-60	45-146	90-240	.00-.03	.00-.06	.02-.10

103.8 RAT MUSCLE CELLS HAVE AN ENDOGENOUS ELECTRIC FIELD FOCUSED AT THE SYNAPSE. W.J. Betz and J.H. Caldwell. Dept. of Physiology, Univ. of Colorado Medical School and Dept. of Mol. and Cell. Biol., National Jewish Hospital & Research Center, Denver, CO.

Using a vibrating microelectrode (Jaffe & Nuccitelli, J. Cell Biol., 63:614, 1974), we have been able to measure a steady endogenous current generated by rat skeletal muscle cells. This d.c. current leaves from the synaptic region and reenters the cell in the flanking extrajunctional regions. In other words, the cell creates an electric field around itself with an anodal region focused at the synapse and neighboring cathodal regions on either side of the synapse.

This steady current could arise from an uneven distribution of ion pumps or ion channels along the length of the muscle cell. The only known nonuniformity along the length of the cell is the acetylcholine receptor, but the d.c. current is unaffected by alpha bungarotoxin or curare. Therefore the acetylcholine channel is not required for the current.

Electrogenic Na/K pumps are present in rat muscle. If these pumps were concentrated near the synapse, they could create the d.c. current. When acetylcholine (ACh) was added to the bath, a large inward current was measured at the synapse due to entry of sodium into the cell. After the ACh was removed, the outward current near the synapse was transiently much larger than normal. This transient increase was blocked by low concentrations of ouabain (10-100 μM) and is attributed to the activation of electrogenic Na/K pumps in the synaptic region. The steady endogenous current, however, was not blocked by large concentrations of ouabain (10mM). Therefore the endogenous current is not due to increased activity of electrogenic Na pumps in the synaptic region.

It can be calculated from our measurements of extracellular currents that the membrane at or near the synapse is about 0.5-1mV hyperpolarized relative to the extrajunctional membrane. Any distribution of ion pumps or channels that would cause this hyperpolarization of the synaptic membrane will generate the observed current pattern. For example, either a reduced sodium conductance or an increased potassium conductance near the synapse would generate the current. Chloride ions could also generate the current if they are not passively distributed. We next present evidence that chloride is pumped into the rat lumbrical muscle and that chloride ions should also be considered as a cause of the endogenous field.

103.9 A CHLORIDE PUMP IS PRESENT IN A MUSCLE WITH AN ENDOGENOUS ELECTRIC FIELD. S. Kinnamon*, W.J. Betz, and J.H. Caldwell (SPON: R. Mains). Dept. of Physiol., Univ. of Colo. Med. Sch. and Dept. of Mol. and Cell. Biol., National Jewish Hosp. & Res. Center, Denver, CO.

The endogenous electric field around rat muscle cells is focused at the synapse and reflects a nonuniform distribution of ion channels or pumps. If Cl^- is not passively distributed, a nonuniform Cl conductance along the cell could create the electric field. We present evidence here that the muscle contains a Cl^- pump which transports Cl^- into the cell.

If the membrane potential (Vm) is recorded with an intracellular electrode and the saline is replaced with a Cl^- free medium, the cell undergoes a transient depolarization. After the transient depolarization, the potential returns to a stable value which is hyperpolarized (~10mV) compared to the original membrane potential. If Cl^- were passively distributed, Vm should have returned to its original value. Drugs (furosemide, 10 μM, and bumetamide, .25 μM) which are reported to block Cl^- transport in other tissues cause a membrane hyperpolarization. Finally, a monocarboxylic acid (A9C) which blocks G_{Cl} in rat muscle (Palade & Barchi, J. Gen. Physiol., 69:879, 1977) also causes a hyperpolarization. All of these experiments suggest the existence of a Cl^- pump which pumps Cl^- into the cell.

It has been shown in other tissues that the Cl^- pump is a Na/Cl cotransport system. Therefore we also tested the effect of Na removal. When the cell is shifted to a medium with Na replaced by choline, the membrane potential hyperpolarizes by 10-15mV. This is much larger than one would predict from the Goldman equation and the permeability ratio of Na to K (Robbins, J. Physiol., 271:605, 1977), suggesting a dual effect of Na substitution on Vm. We tested this by testing the effects of Na removal in muscles in which the Cl pump had previously been blocked with furosemide. In this case Vm hyperpolarizes by only a few mV, which is consistent with the amount predicted by the Goldman equation. Thus Na removal appears to block an inwardly directed Cl pump.

The endogenous d.c. current (described in the preceding abstract) was measured with the vibrating probe under the same conditions. Zero external Cl^- or Na, furosemide, and A9C each eliminated the d.c. current. This, however, did not permit us to distinguish between Cl^- and K^+ because the driving force for K^+ is decreasing at the same time that Cl^- movement is decreasing. These experiments did allow us to rule out nonuniform Na channel distribution as the cause of the endogenous current for the following reason: All of the above experiments cause the cell to hyperpolarize which increases the driving force upon sodium and therefore should increase any current due to Na. The endogenous current measured by the vibrating probe, however, decreased.

The endogenous field could be due to either an abundance of K^+ channels or to a reduction of Cl^- channels near the synapse. Experiments described in the next abstract allowed us to choose between these models.

103.10 NONUNIFORM CHLORIDE CONDUCTANCE ALONG THE LENGTH OF A MUSCLE CELL: BASIS OF ENDOGENOUS ELECTRIC FIELD. G. Harris*, J.H. Caldwell, and W.J. Betz (SPON: M. Dubin). Dept. of Physiol., Univ. of Colo. Med. Sch. and Dept. of Mol. and Cell. Biol., National Jewish Hosp. and Research Center, Denver, CO.

An endogenous electric field around rat muscle cells has been described in the last two abstracts. The field is focused at the synapse with current (defined conventionally as the direction of positive charge flow) leaving the cell in the synaptic region. This field and associated current could be due to a nonuniform distribution of ion channels or pumps and the object here is to determine the important ion(s). The field is not dependent upon activation of ACh receptors, electrogenic Na/K pumping, or the resting sodium conductance. It is not altered by changes in external calcium or external pH. The distinction between a nonuniform K^+ or Cl^- conductance is made here.

Three experiments cause a change in the current (measured with a vibrating microelectrode) that cannot be explained by a dependence upon K^+. These experiments are (1) returning to normal Na from zero external Na, (2) zero external K^+, and (3) Ba^{+2} (50 μM). Each of these causes a reversal of the extracellular current and each of them depolarizes the membrane. This depolarization could reverse the driving force for Cl but not for K^+. These experiments imply that Cl^- is the most likely candidate for the generation of the endogenous current and field.

The hypothesis that Cl^- channels are unevenly distributed along the length of the cell was tested directly. The muscle was bathed in zero Cl and the membrane potential was measured with an intracellular electrode. A second electrode containing Cl^- was placed outside the cell, and was used to apply brief pulses of Cl^- to a localized patch of membrane. The entry of Cl^- caused a transient hyperpolarization, which provided a measure of G_{Cl} in the region of the cell that was superfused. Responses in the synaptic region, compared to those in the extrajunctional region, were significantly smaller, indicating a reduced G_{Cl} near the synapse. A similar experiment performed with pulses of elevated K^+ showed no difference in G_K along the cell.

We conclude that the simplest explanation for the endogenous electric field and the currents associated with this field is a nonuniform Cl^- conductance. A greater permeability to Cl^- in the extrajunctional regions than at the synapse would generate the observed field. The possible significance of this field for nerve-muscle interactions and for muscle development is discussed in the next abstract.

103.11 SPECULATIONS ON THE SIGNIFICANCE OF THE NONUNIFORM CHLORIDE CONDUCTANCE AND THE ENDOGENOUS D. C. ELECTRIC FIELD. J.H. Caldwell, W.J. Betz, S. Kinnamon*, and G. Harris*. Dept. of Physiol., Univ. of Colo. Med. Sch. and Dept. of Mol. and Cell. Biol., National Jewish Hospital and Res. Center, Denver, CO.

We have shown in the previous abstracts that rat muscle has a reduced chloride conductance near the synapse and that this is the basis of the endogenous steady electic field around the muscle cell. There is evidence that the chloride conductance of rat plasma membrane is preferentially located in the transverse tubular membrane (Dulhunty, J. Memb. Biol., 45:293-310, 1979). Hence, one very simple explanation (hypothetical at present) for the nonuniform chloride conductance is that the muscle fiber has less transverse tubular membrane near the neuromuscular junction. What would be the consequence of a reduction in transverse tubule membrane near the synapse? It can be shown that the result is to increase the input impedance for high frequencies (e.g. an epp) while not increasing the input impedance for low frequencies, which can lead to myotonia.

The above hypothesis provides no role for the field and it is worth speculating whether or not this field could be useful. There are several ways in which this field might have some significance for nerve-muscle interaction or for postsynaptic membrane specializations. It has been shown that an imposed electric field will direct neurite growth (Jaffe and Poo, J. Exp. Zool., 209:115-128, 1979). A denervated as well as innervated muscle has an electric field surrounding each cell. This field might affect the growth of regenerating axons or the growth of sprouting axons in a partially denervated muscle.

Imposed electric fields can cause lateral movement of membrane proteins (Poo and Robinson, Nature, 265:602-605, 1977). Since the endogenous field is steady and is focused at the synapse, it potentially could move membrane proteins (e.g. acetylcholine receptors) toward or away from the synapse.

All of these speculative ideas remain to be tested.

103.12 STRETCH INDUCED BLOCK OF NERVE POTENTIALS. J.L. Johnson. USD School of Medicine, Dept. of Physiol. & Pharmacol., Vermillion, SD 57069

Nerve stretch has been shown to produce a beading of axons in vertebrate nerves (Exp. Neurol., 12, 1965, 84), but the effects of such conditions on action potential generation and conduction remain to be explored. Therefore, the effects of nerve stretch upon Aα nerve potential amplitude and conduction of latency was measured in the frog sciatic nerve. The magnitude of stretch forces applied ranged from 0-20 grams. The latency increased linearly with the stretch force applied ($r = 0.989$, $P < 0.01$), while the square of the Aα nerve potential amplitude correlated expotentially with this stretch force ($r = 0.989$, $P < 0.01$). Minimal recovery was observed from the stretch effects within 30 seconds after removal of the weight. For both low and high amounts of weight used, on the other hand, complete recovery was noted after 10 minutes ($P < 0.001$). Such a stretch block and recovery sequence could be repeatably observed in the same nerve preparation. Smaller nerves were more sensitive to a given stretch force than the nerves from larger frogs. In addition, nerves from frogs suffering severe energy depletion with muscular wasting, were exquisitely sensitive to the blocking effects of stretch, even though recovery from the stretch effect was the same after 10 min. Comparison of the above data for the frog sciatic nerve, with the stretch effects on conduction in the giant axons of the crayfish and earthworm ventral nerve cord confirm the size principle (nerve weight per unit length) for stretch block effectiveness. This suggests that it is the volume of nerve connective tissue rather than the axon size per se which determines the resistance to the blocking effects of stretch. Nerve conduction was reversibly blocked using weights which were 500-5000X the nerve weight. The nerve structure would seem to be such that tremendous stretch forces could be endured to result in only minimal nerve fiber damage, as long as the elastic limits are not exceeded. Any elongation of the nerve during stretch would also require a remarkable resiliency of axoplasmic structure, since these effects were consistently reversible. It would seem that nerve stretch could be another pathophysiological variable, together with pressure effects, in leading to altered nerve conduction particularly in states of energy depletion with tissue wasting. (Supported by institutional funds from the University of South Dakota School of Medicine).

103.13 INCORPORATION OF TRANSITION METAL IONS INTO CRAB NERVE FIBERS.
K. Iwasa*[1], T. Inubushi*[2], and I. Tasaki[1]. Lab. of Neurobiology[1], NIMH, Bethesda, MD 20205 and Lab. of Chemical Physics[2], NIADDK, Bethesda, MD 20205.

It was reported [Dea et al., Science 175(1972)206] that addition of paramagnetic cations, e. g. Mn^{2+} and Co^{2+}, at low concentrations to the external medium could be used to distinguish extracellular water from intracellular water, since the paramagnetic ions broaden and shift the peak of the extracellular water signal in proton magnetic resonance (PMR) spectra. It was also reported [Fritz & Swift, Biophys. J. 7(1967)675] that the number of water molecules in the nerve during potassium depolarization could be determined by examining the water peak in the PMR spectra in the presence of these paramagnetic ions, assuming that these ions did not penetrate the nerve membrane. We have examined the reliability of this method of studying water and ion movements across the nerve membrane.

We found that nerve fibers of the blue crab, *Callinectes sapidus*, swell when immersed in solutions rich in K^+, Cs^+, or Rb^+, as well as in artificial seawater to which veratridine (200 μm) is added. This slow swelling, as distinguished from the rapid swelling which takes place during the time course of a single action potential, is dependent on the anion species present in the external medium. The efficiency of anions present in the external medium in causing the slow swelling is in the order: I^-, SCN^-, Br^-, Cl^-, F^-, and glutamate, I^- being the most effective. The slow swelling is caused by the influx of salts accompanied by water molecules. We found by using PMR and radioactive tracer methods that the paramagnetic anions are also incorporated into nerve fibers, and that the PMR signal of the intracellular water is also modified by these paramagnetic ions. When the concentration of Mn^{2+} added to a K-rich medium is 5 mM, the internal Mn^{2+} concentration reaches 1 mM in one hour; when 5 mM Mn^{2+} is added to normal artificial seawater outside, the internal Mn^{2+} concentration reached is about 0.1 mM in the same time period. The PMR method gives useful information about the distribution of paramagnetic ions in the nerve fibers rather than distinguishing intracellular and extracellular water.

104 SYMPOSIUM. MOLECULAR AND GENETIC STUDIES OF THE VOLTAGE-DEPENDENT SODIUM CHANNEL. L.M. Hall, Albert Einstein College of Medicine (Chairman); S.R. Levinson*, Univ. of Colorado School of Medicine; L.C. Fritz, Calif. Institute of Technology; W.A. Catterall, Univ. of Washington; B. Ganetzky*, Univ. of Wisconsin, Madison.

The voltage-dependent sodium channels are involved in conduction of action potentials in nerve and muscle. Much of our understanding of these sodium channels at the molecular level has come from studies involving neurotoxins such as tetrodotoxin and saxitoxin which exhibit high affinity binding to the channel. In this symposium three different approaches to the study of sodium channels will be discussed. Questions concerning the biochemical nature of the channel have been approached by purification of toxin-binding components from the electric organ of the eel Electrophorus electricus and from rat brain. Data concerning the subunit composition, amino acid analysis, carbohydrate composition and physical properties of material purified from different sources will be compared. A second approach has involved the production of monoclonal and polyclonal antibodies which recognize the voltage-dependent sodium channel. These immunoreagents are able to precipitate the saxitoxin-binding component from crude preparations of detergent-solubilized membranes. The properties of these antibodies as well as their use in the cytochemical localization of sodium channels will be discussed. Finally, genetic strategies for isolation of mutations affecting sodium channels and related processes will be presented. Using specific neurotoxins as selective agents, neuroblastoma clones have been isolated which are lacking functional sodium channels and fail to synthesize polypeptides identified in purification studies as channel constituents. In studies on Drosophila, mutations that cause temperature-induced paralysis appear to affect sodium channels. Selection for second site mutations that interact with the paralysis-inducing mutations provides a strategy for identifying other gene products that are involved in the same physiological pathway of cell excitability. The combination of biochemical, immunological and genetical approaches should allow the molecular dissection of structural and functional aspects of voltage-dependent sodium channels.

105 SYMPOSIUM. THE PRIMATE FRONTAL LOBES: MECHANISMS FOR THE ORDERING OF COMPLEX BEHAVIOR. Michael E. Goldberg, Lab. of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20205 (Chairman); Brenda A. Milner, McGill Univ., Quebec; Patricia Goldman-Rakic, Yale Univ. School of Med.; George R. Leichnetz, Med. College of Virginia; Kisou Kubota, Kyoto Univ., Japan.

The frontal lobes are the largest part of the cerebral cortex in the human, and the part which has developed most strikingly during evolution. The frontal cortex is not involved in simple perception or the elaboration of uncomplicated motor behavior, since monkeys or humans with frontal lesions can appear normal to superficial examination. Thus a great curiosity of nineteenth century medicine was Phineas Gage, a man who spent many years of his life with a crowbar imbedded in his frontal lobe, and a tragedy of twentieth century medicine was the large number of psychiatric patients in whom frontal lobotomies were performed for therapeutic reasons.

Yet it has been clear that frontal lesions produce deficits not in the execution of the movements for a task, but rather with the determination of when, if at all, to perform a task well within the motor abilities of the subject. A seminal finding was that of Jacobsen who showed that rhesus monkeys with frontal lesions have difficulty performing tasks in which they must make a movement after a delay.

In this symposium we will describe the results of new approaches into the question of how the frontal lobes participate in the ordering of complex motor behavior. After a brief historical introduction, Brenda Milner will describe deficits exhibited by patients who have undergone limited frontal ablations for intractable epilepsy. These patients have difficulty with performing sequential self-ordered tasks, or making eye movements away from salient visual stimuli. Patricia Goldman-Rakic will describe the results of 2-deoxyglucose studies to map those areas of the monkey frontal lobe active during the performance of a delayed response task. She will also describe the anatomical connections of these areas. George Leichnetz will describe the efferent connections by which the frontal cortex can influence the subcortical oculomotor system of the rhesus monkey. Michael Goldberg will describe the cellular activity of the monkey frontal eye fields that precedes the generation of a rapid eye movement by a rhesus monkey, and address the question of what eye movements are associated with activity in the frontal eye fields, and what are not. Kisou Kubota will describe the activity of prefrontal neurons in a visually guided arm movement task in the rhesus monkey, in order to show how such neuronal activity could be important in the neural events associated with complicated motor behavior.

106.1 MORPHOLOGICAL BASIS OF LONG-TERM HABITUATION AND SENSITIZATION IN APLYSIA. C.H. Bailey* and M. Chen. Center for Neurobiol. & Behav., Depts. Anat., Physiol., and Psychiat., Columbia Univ., P & S, and the N.Y.S. Psychiatric Instit., N.Y., N.Y. 10032.

We have begun to explore the morphological basis of the prolonged synaptic plasticity that underlies long-term habituation and sensitization of the gill-withdrawal reflex in Aplysia californica. Toward this end we have examined the morphology of identified sensory neuron synapses (the critical site of plasticity for the short-term form of both types of learning) in control and behaviorally-modified animals.

Following behavioral training (Carew et al., 1972; Pinsker et al., 1973), sensory neurons in the abdominal ganglia of control (untrained), long-term habituated and long-term sensitized animals were injected with horseradish peroxidase. A total of 3 cells in control animals, 5 in habituated and 6 in sensitized were successfully injected and reacted. Ganglia were thick-sectioned until a region rich in labeled sensory neuron terminals was encountered. Serial thin sections were cut and every HRP-labeled profile in each section photographed. Sensory neuron varicosities were then reconstructed and analyzed following a blind procedure. A total of 311 completely reconstructed varicosities taken from two animals in each behavioral group were analyzed.

We have compared the frequency of active zones in sensory neurons in the three groups of animals. We found that the ratio of active zones to varicosities increases from 12% (13/111) in habituated animals to 41% (38/93) for control animals to 65% (70/107) in sensitized animals. In addition, we have found a parallel increase in both the size of sensory neuron active zones and the total number of vesicles associated with each release site. These values range from a mean active zone surface area of $0.127 \mu m^2 \pm 0.02$ (S.E.M.) occupied by 6 vesicles for habituated animals to $0.197 \mu m^2 \pm 0.02$ and 13 vesicles for control and $0.344 \mu m^2 \pm 0.03$ and 22 vesicles for sensitized animals. The packing density of vesicles at active zones in control and sensitized animals is approximately the same (65 vesicles/μm^2) suggesting that the increase in vesicle number found at sensitized active zones is merely a reflection of the increased area available for vesicle loading. In contrast, the packing density in habituated synapses is only 41. This may reflect additional structural differences in the synapses of habituated animals. Consistent with this notion we have observed that the varicosities of habituated animals routinely contain synaptic vesicles with irregular contours that frequently appear to fuse forming cisternae.

This study has shown that clear structural changes accompany long-term behavioral modifications and can be detected at the level of those synapses critically involved in the behavior. Our results suggest that learning may modulate synaptic effectiveness by altering the number and/or size of active zones and their vesicle complement. These new morphological features could represent an anatomical substrate for memory consolidation.

106.2 EVIDENCE FOR A HETEROSYNAPTIC COMPONENT OF HABITUATION IN APLYSIA. J.I. Goldberg* and K. Lukowiak*. (SPON: W. Ruwe). Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

In Aplysia, the defensive gill withdrawal reflex (GWR) and siphon withdrawal reflex (SWR) can both be evoked by punctate tactile stimulation of the siphon, gill or mantle. Repeated stimulation at any of these sites results in reflex habituation, which is the product of a frequency-dependent decrease in transmitter release from the sensory and motor neurons that comprise the central reflex arcs. In this study, we examined whether habituation may also be mediated by heterosynaptic depression of the synapses made by the sensory neurons. That the GWR and SWR both displayed transfer of habituation is evidence in support of this hypothesis. Transfer of habituation is a decrement in the amplitude of the reflex evoked at one stimulation site subsequent to habituating the reflex with repeated stimulation at another site. Such a transfer of suppressive information likely involves heterosynaptic mechanisms which may also operate during habitation itself. To demonstrate heterosynaptic depression, a semi-intact preparation consisting of the siphon, gill, mantle and abdominal ganglion (CNS) was used. A siphon mechanoreceptor (LE cluster) and either a gill motor neuron, siphon motor neuron or non-motor follower neuron were simultaneously impaled with intracellular microelectrodes. Synaptic transmission was evaluated by measuring the EPSPs evoked by single LE action potentials. When two such trials were separated by 20 min, there was no change in evoked EPSP amplitude. However, when a third trial (ISI = 20 min) was preceded by a series of ten tactile stimuli (ISI = 30 s) presented to the siphon at a site apart from the sensory field of the impaled mechanoreceptor, the amplitude of the evoked EPSP was reduced by approximately 45% (n = 5). A subsequent novel stimulus, such as a tactile gill stimulus, partially augmented the EPSP evoked 30 - 60 s later in only half of the experiments. Similar results were obtained when the repeated tactile stimuli were presented to the gill. Moreover, the results did not vary according to the type of follower cell. These results suggest that repeated excitation of a reflex pathway activated an inhibitory interneuron(s) or modulatory neuron(s) that subsequently depressed synaptic transmission at naive first-order synapses. Such a mechanism could underlie transfer of habituation. Furthermore, since all the first order synapses were affected similarly, it is likely that the heterosynaptic depression also contributes to response decrement in the primary habituation pathway.

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106.3 REWARD LEARNING IS PROCESSED SLOWLY IN DROSOPHILA. Nancy Bonini*, Bruce Tempel, and William Quinn*. (SPON: Roger Cholewiak). Department of Biology, Princeton U., Princeton, N.J. 08544

The fruit fly, Drosophila melanogaster, can learn a variety of negatively reinforced tasks. We have reported a positively reinforced paradigm in which hungry flies associate a food reward with an odor cue. We showed that memory lasted much longer after 1.0 M sucrose reward than after similar training with 90 V electric shock (Tempel and Quinn, 1980, Neurosci. Abst. 6: 589). Here we provide two pieces of evidence supporting the suggestion that memory is processed more slowly after sucrose than after shock conditioning in these paradigms. First, over a wide range of reinforcer magnitudes, memory lasts longer after sucrose than after shock. Second, memory is consolidated into a cold-anesthesia resistant form later after sucrose reinforcement than after shock.

To measure memory at lower magnitudes of reinforcement, we first determined learning ability over a range of reinforcer magnitudes. Both sucrose and shock reinforced learning decreased gradually with decreasing magnitudes of reinforcement. Near threshold levels for detection--0.004 M sucrose and 5 V shock--learning levels fell dramatically. This suggests flies were able to learn provided they could sense the reinforcement. Although learning was lower with low magnitudes of reinforcement, memory decay rates were unchanged--characteristically slow for sucrose learning and rapid for shock learning. Using either 90 V or 10 V shock, memory disappeared by 4 hours after training. In contrast, performance was still strong 6 hours after training with either 1.0 M or 0.025 M sucrose.

Cold anesthesia, applied soon after training, disrupts memory formation in many animals. Later, without further training, memory becomes immune to anesthesia. By cooling flies at various times before and after training, we have studied this consolidation process, the transfer of memory from short-term, labile to long-term, stable memory phases. Regardless of whether flies received 10 cycles of shock training to a single odor or 2 cycles of two-odor discriminative training, memory was consolidated between 10 and 30 minutes after negatively reinforced training. In contrast, memory after sucrose training remained disruptible by cold for 90-120 minutes.

These results suggested that consolidation and final decay of memory occurred more slowly after sucrose than after shock conditioning. This suggestion was supported by results from memory mutants.

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106.4 DEFECTIVE ADENYLATE CYCLASE IN THE DROSOPHILA LEARNING MUTANT, RUTABAGA. Margaret S. Livingstone, Patricia P. Sziber* and William G. Quinn*. Dept. of Biology, Princeton University, Princeton, New Jersey 08544.

Several single-gene mutations in Drosophila produce remarkably selective defects in associative learning. The learning mutant, dunce, has previously been shown to lack one of two forms of the enzyme cyclic AMP phosphodiesterase.[1] dunce flies are in addition female-sterile. Here we report that another X-chromosome mutation, also originally isolated as a learning mutant, has altered abdominal adenylate cyclase activity.

rutabaga abdominal adenylate cyclase is different from the wild-type enzyme in three ways: 1) the rutabaga enzyme has a three-fold lower substrate affinity than does the wild-type enzyme; 2) rutabaga/wild-type heterozygotes show biphasic kinetics identical to what would be obtained for a mixture of the two parental types of enzyme; 3) the rutabaga enzyme is more thermolabile than the wild-type enzyme. These results are consistent with the possibility that the rutabaga mutation is in the structural gene for some part of the adenylate cyclase enzyme.

The biochemical effects of the rutabaga mutation are not equally apparent in all tissues. Most, if not all, of the enzyme in the abdomen is altered by the rutabaga mutation, but less than 30% of the enzyme from either the head or the thorax is affected by the mutation. Therefore either rutabaga is a tissue-specific mutation or there are at least two forms of the enzyme, one of which is the predominant species in the head and thorax and the other, affected by the rutabaga mutation, is the predominant form in the abdomen.

Since the two learning mutations, dunce and rutabaga, both directly affect cyclic AMP metabolism, but in opposite directions, we tested the effect of putting the two mutations in the same fly. Double mutants ($dunce^{M11}$, rutabaga) did not learn better than either single mutant, but the rutabaga mutation attenuated the increase in endogenous cyclic AMP levels caused by the dunce mutation. Furthermore, the rutabaga mutation suppressed the female-sterility associated with the dunce mutation.

Thus two mutations, isolated because they fail to learn, have turned out to directly affect cyclic AMP metabolism. Another mutant Ddc^2 which decreases the synthesis of dopamine and serotonin[3] and therefore indirectly affects cyclic AMP metabolism, also fails to learn.[4]

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106.5 MUTATIONS IN THE DOPA-DECARBOXYLASE GENE AFFECT LEARNING BUT NOT MEMORY IN DROSOPHILA. Bruce L. Tempel and William G. Quinn*. Department of Biology, Princeton University, Princeton NJ 08544

When shifted to a restrictive temperature, the Ddc^{ts1} mutation blocks dopamine and serotonin as well as blocking both appetitively and aversively reinforced associative learning (Tempel and Livingstone, 1981, Neurosci. Abstr. 7: 351). We report here that a second temperature-sensitive allele of Ddc, Ddc^{ts2}, had similar effects on learning. Using Ddc stocks with intermediate effects on enzyme activity and learning ability, we find no effect of the Ddc mutation on memory decay rate.

Geneticists worry that the phenotypes they observe might result from non-specific genetic background effects in a particular stock of flies. To avoid this objection, Wright isolated several temperature-sensitive alleles of the Ddc gene and determined that the gene affected enzyme activity. Using these mutants, we tested the correlation between behavior and nervous system enzyme activity. When adult Ddc^{ts1} or Ddc^{ts2} flies are shifted to a restrictive temperature for three days, they do not synthesize dopamine or serotonin and cannot learn. This suggests that the Ddc gene itself affects learning.

In order to study memory in the Ddc mutants, we chose two stocks with intermediate synthetic activities and learning abilities. Comparing mutants to normal flies, memory decay is unaffected; both wild-type and Ddc flies forget electric shock training within 4 hours. Consistent with other reward learning studies, memory is still strong in both normal and mutant flies 6 hours after sucrose reinforced training. Apparently, once learning is established, memory is unaffected and contrasts dramatically with the normal learning but abbreviated memory periods siin in mutants (dunce and rutabaga) that cannot regulate cyclic AMP levels.

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106.6 ASSOCIATIVE LEARNING IN THE ISOLATED SIPHON, MANTLE, GILL AND ABDOMINAL GANGLION PREPARATION OF APLYSIA: A NEW PARADIGM. Ken Lukowiak* (Spon: R. Berry). Dept. of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

The siphon, mantle, gill and abdominal ganglion preparation of Aplysia has proved to be an excellent system in which to study the neural mechanisms which underlie forms of non-associative learning (habituation and sensitization) and, more recently, associative learning (classical conditioning). However, using the Lukowiak & Sahley (1981) paradigm, it has not been possible to analyze the neural mechanisms of the associative learning.

To maximize the possibilities of uncovering the neuronal mechanisms of associative learning, a different paradigm was constructed which makes use of the known neural circuitry of the GWR. The CS was a very weak (50 mg) tactile stimulus delivered to the siphon by the tapper, while the UCS was a stronger, train of tactile stimuli (1.5 g, 8/sec for 2 sec) delivered to the gill by a second tapper. In the experimental group, the CS preceded the UCS by 1 second and the intertrial interval was 2 min. Initially, the CS evoked only a small siphon withdrawal response (SWR) and no observable GWR. In 7 of 9 preparations, however, a GWR was evoked by the CS as early as T_{10} and GWR amplitude progressively increased so that by T_{40} the GWR amplitude evoked began to approach the minimal response criterion set out by Carew et al., 1979. In these preparations, the response was extinguished within 10 presentations of the CS alone but the CS came again to evoke a GWR with as few as 5 additional pairings. In these same preparations, the amplitude of the SWR also increased steadily over the course of the experiment. In control group preparations the CS alone was presented. The small SWR habituated rapidly and no GWR was evoked. In a second control group, the UCS was presented at some random interval after the presentation of the CS, the CS never came to evoke a GWR. In a third control (n = 3), the UCS was initially presented at some random interval and after 40 trials the CS still did not evoke a GWR. However, following forward pairing (35 trials), the CS evoked a small GWR. Because so much of the neuronal circuitry which underlies the GWR evoked by tactile stimulation of the siphon is known, there is an excellent possibility that the neuronal mechanisms which underlie the associative learning can be worked out.

Supported by MRC.

106.7 DIFFERENTIAL CLASSICAL CONDITIONING OF A DEFENSIVE WITHDRAWAL REFLEX IN APLYSIA. T. J. Carew, R. D. Hawkins, and E. R. Kandel. Center for Neurobiology & Behavior, Depts. Physiol., Neurol. & Psychiat., P & S, Columbia Univ., and N.Y. State Psychiatric Institute, New York, N.Y. 10032.

The gill and siphon withdrawal reflex of Aplysia shows aversive classical conditioning with a weak tactile stimulus to the siphon as the conditioned stimulus (CS) and a strong electrical shock to the tail as the unconditioned stimulus (US) (Carew, Walters and Kandel, 1981). We now report that this reflex is capable of differential conditioning with two conditioned stimuli applied to different sites, the mantle shelf and the siphon. Since each animal serves as its own control, this form of discriminative learning provides a powerful behavioral tool for a cellular analysis of classical conditioning.

To examine differential conditioning we trained two groups for 15 trials with an inter-trial interval of 5 min. One group (SIPHON-PAIRED, N=12) received the siphon CS specifically paired with the tail shock (US) and the mantle CS specifically unpaired. The other group (MANTLE-PAIRED, N=12) received the mantle CS paired and the siphon CS unpaired. Each pathway was pre-tested prior to training. Thirty minutes after training, SIPHON-PAIRED animals showed significantly longer siphon withdrawal to the siphon CS than to the mantle CS ($p < .05$), while MANTLE-PAIRED animals showed significantly longer responses to the mantle CS than to the siphon CS ($p < .01$). Differential conditioning was evident both immediately and 24 hrs after even a single training trial (N=21) as well as after 5 trials (N=23) and 15 trials (N=24) ($p < .005$ in each case). We also examined the relation between sensitization and differential conditioning in another experiment (N=24). We gave one pathway 5 trials of paired stimulation, while the other pathway received no stimulation. Both immediately and 24 hrs after training, the unstimulated pathway exhibited significant sensitization ($p < .01$), but the conditioned pathway exhibited an even greater increase in responding ($p < .005$).

The afferent input from the mantle and siphon is carried by two identified clusters of sensory neurons: the RE and LE clusters. In a final experiment (N=16) we examined whether we could differentially condition sub-populations within a single (LE) cluster of sensory neurons. Two sites on the siphon were stimulated with implanted electrodes as paired and unpaired inputs. After 5 trials the paired site showed significant differential conditioning compared to the unpaired site ($p < .005$).

The fact that differential conditioning can be produced in an elementary reflex mediated by relatively few cells demonstrates that this advanced form of learning is not an exclusive feature of behaviors with complex neural circuitry. Moreover, the finding that two independent sensory inputs that activate a common set of interneurons and motorneurons can be differentially conditioned, greatly restricts the possible cellular loci where the associative changes must occur.

106.8 ACTIVITY-DEPENDENT FACILITATION ACCOUNTS FOR PAIRING SPECIFICITY IN CLASSICAL CONDITIONING OF A DEFENSIVE WITHDRAWAL REFLEX IN APLYSIA. T. W. Abrams, R.D. Hawkins, T. J. Carew, and E. R. Kandel. Center for Neurobiology & Behavior, Depts. Physiol., Neurol. & Psychiat., P & S, Columbia Univ., and N.Y. State Psychiatric Institute, New York, N.Y. 10032.

Pairing specificity is a fundamental feature of classical conditioning. We have explored the cellular mechanism for pairing specificity using differential conditioning of the siphon withdrawal reflex in Aplysia (Carew, Hawkins and Kandel, 1982). In this paradigm, tactile conditioned stimuli (CSs) are presented to two different sites: one is specifically paired with a tail shock, the unconditioned stimulus (US), the other is specifically unpaired. Following training the response to the paired CS is preferentially enhanced compared to the response to the unpaired CS. Thus either of two sensory inputs to a common motor system can be selectively facilitated as a function of temporal pairing with the US.

We investigated the possibility that pairing specificity might be due to activity-dependent facilitation (Kandel and Tauc, 1965). According to this hypothesis, the US facilitates synaptic transmission in both populations of sensory neurons, but the facilitation is enhanced in those sensory neurons that fire action potentials just prior to the delivery of the US. To test this hypothesis, we attempted to differentially facilitate the monosynaptic EPSPs produced by two siphon sensory neurons in a common postsynaptic interneuron or motoneuron. Activity in one of these sensory neurons was paired with the US (a shock to the tail or to the tail nerves), and activity in the other sensory neuron was specifically unpaired. In another set of experiments, one sensory neuron was paired and the other was not activated during training, serving as a US alone control. To compare the effects of training in different sensory neurons, EPSP amplitudes were expressed as percent of pretraining amplitude. The EPSPs from the paired sensory neurons were significantly facilitated (206%) compared to the EPSPs from either the specifically unpaired cells (92%) or the cells that received the US alone (123%) ($p < .005$, and $p < .05$, respectively; N=22 experiments). Thus, activity in the sensory neurons immediately preceding the US significantly enhances the conventional facilitation of synaptic transmission produced by the US in these neurons.

These results are strikingly similar to behavioral results obtained with an identical training protocol (paired=201%, unpaired=99%, and US-alone=110%, Carew et al., 1982). This congruence between the results obtained from sensory cells mediating the behavior and the behavior itself suggests that activity-dependent enhancement of facilitation accounts importantly for the pairing specificity of associative learning in this reflex.

106.9 MECHANISM OF ACTIVITY-DEPENDENT FACILITATION UNDERLYING CLASSICAL CONDITIONING IN APLYSIA. R. D. Hawkins, T. W. Abrams, T. J. Carew, and E. R. Kandel. Center for Neurobiol. & Behavior, Depts. Physiol., Neurol., and Psychiat., P & S, Columbia Univ., and N. Y. State Psychiatric Institute, New York, N. Y. 10032.

The withdrawal reflex of Aplysia exhibits differential conditioning in experiments using tail shock as the unconditioned stimulus and using as discriminative stimuli either stimulation of the siphon and mantle shelf or stimulation of two points on the siphon (Carew, Hawkins and Kandel, 1982). Employing a similar experimental protocol, we found that monosynaptic EPSPs from two siphon sensory neurons to a common follower neuron exhibit differential facilitation that can account quantitatively for the behavioral conditioning (Abrams, Hawkins, Carew and Kandel, 1982). Differential facilitation of EPSPs could result from either a presynaptic or a postsynaptic mechanism. Behavioral sensitization of this reflex is due to presynaptic facilitation at the same sensory neuron synapses (Castellucci and Kandel, 1976). The facilitation is in turn due to an increase in Ca^{++} current that is manifest as an increase in the duration of action potentials in the sensory neurons in TEA solution (Klein and Kandel, 1978). We therefore used this measure to investigate the possibility of a presynaptic mechanism of conditioning. We placed the isolated central nervous system in 50 mM TEA, left the tail attached for a US input, and stimulated two sensory neurons intracellularly (as CS inputs), causing them to fire action potentials once every 5 minutes. The action potentials in one sensory neuron were paired with tail shock, while action potentials in the other neuron were specifically unpaired. Five to 15 minutes following 15 training trials, broadening of the spike in the paired sensory neuron was significantly greater than broadening of the spike in the unpaired neuron ($\bar{x}$ = 123% for the paired neuron and 98% for the unpaired neuron in 21 experiments, $p < .01$). This difference was maintained for at least 3 hours after training, indicating that the mechanism for pairing specificity and at least the short-term memory for the learning share a common locus, the terminals of the sensory neurons. Moreover, the action potentials in both neurons continued to broaden during the period following training, which parallels behavioral results (Carew, Walters and Kandel, 1981; Carew, Hawkins and Kandel, 1982).

These data indicate that a cellular mechanism of classical conditioning of the withdrawal reflex is simply an extension of the mechanism of sensitization of the reflex: presynaptic facilitation due to an increase in Ca^{++} current during each action potential in the sensory neurons. The pairing specificity characteristic of classical conditioning is due to the fact that this presynaptic facilitation is amplified by preceding spike activity in the sensory neurons. By contrast, preliminary results indicate that spike activity in the postsynaptic neuron, which Hebb (1949) proposed as the crucial event in conditioning, is neither necessary nor sufficient to produce differential facilitation at these synapses.

106.10 ASSOCIATIVE CONDITIONING OF SINGLE SENSORY NEURONS IN APLYSIA: I. ACTIVITY-DEPENDENT HETEROSYNAPTIC FACILITATION. E.T. Walters and J.H. Byrne. Dept. Physiol. and Cell Biol., Univ. Texas Med. Sch. at Houston, Houston TX 77025

We have used a differential classical conditioning procedure to produce a cellular analog of associative learning in single tail sensory neurons in the pleural ganglion of Aplysia. In each animal (N=10) we examined 3 sensory neurons (which were not activated by the US) and their monosynaptic connections to a tail motor neuron. During training one sensory neuron received the conditioned stimulus (CS+, 9 intracellular suprathreshold pulses) 1 sec before the unconditioned stimulus (US, shock to the tail), the second sensory neuron received the same CS 2 min later (CS-), and the third received no CS, serving as a sensitization control (SENS). 5 training trials were given at 5 min intervals, followed by at least 5 test trials. Each form of training produced significant facilitation of the EPSP, but cells trained with the CS+ showed significantly more facilitation than either the CS- cells ($p < .025$ on every trial except the first) or the SENS cells ($p < .025$ on every trial). Differences between CS+ and CS- cells were sometimes observed for over 75 min after training.

These results show that spike activity paired with sensitizing tail stimulation can dramatically amplify the facilitation normally produced by the tail stimulus. A clue to the underlying mechanisms may be provided by the finding that the CS presented alone produces short-term post-tetanic potentiation (PTP). In many systems PTP has been suggested to be due to Ca^{++} accumulation during the burst of spikes. On the other hand, several lines of evidence suggest the involvement of cAMP in heterosynaptic facilitation in these sensory neurons, as well as in other cells. Thus an attractive hypothesis is that the associative mechanism involves an amplification of the effects of one major cellular regulator (cAMP) by another (Ca^{++}); for example, through a Ca^{++}-sensitive adenylate cyclase, or synergistic Ca^{++}-dependent and cAMP-dependent protein kinases.

Activity-dependent modulation provides an efficient means of selectively addressing a modulatory signal to functionally active cells, and thus might be involved in many forms of learning and plasticity. The general utility of this mechanism is suggested by evidence that it may also underlie a second form of learning in this preparation - like the CS+ cells, sensory cells directly activated by the US showed significantly amplified facilitation. Thus a common associative mechanism may encode both the predictive properties of the CS+ in some cells, and information about the site and quality of the US in others.

106.11 ASSOCIATIVE CONDITIONING OF SINGLE SENSORY NEURONS IN APLYSIA: II. ACTIVITY-DEPENDENT MODULATION OF MEMBRANE RESPONSES. J.H. Byrne and E.T. Walters. Dept. Physiol. and Cell Biol., Univ. of Texas Med. Sch. at Houston, Houston, TX 77025.

Walters and Byrne (this volume) have differentially conditioned single tail sensory neurons in Aplysia, suggesting that activity-dependent heterosynaptic facilitation could be a mechanism for associative learning. We have proposed that this associative mechanism might depend upon an amplification by Ca^{++} of extrinsically modulated cyclic AMP-sensitive processes in the cell. We here describe an effect of pairing on the membrane responses of these cells that may contribute to this associative process.

Pairing an intracellularly activated burst of spikes with tail shock (N=10) depolarized the sensory cell (2.05 $\pm$ 0.38 mV) for 30-300 sec. By contrast, tail shock alone or unpaired shock caused a slow hyperpolarization (1.12 $\pm$ 0.43 and 0.54 $\pm$ 0.20 mV respectively, N=10 in each case), presumably because of lateral inhibition. Paired responses to the shock were significantly depolarized compared to unpaired and shock-alone responses ($P < .005$ in each case). These differences increased with continued training.

Similar results have been obtained by pairing spike activity with bath application of 10^{-4} to 10^{-5} M serotonin (5-HT). The paired responses were depolarized relative to the unpaired responses in 4 of 4 cells examined. In addition, axotomized sensory cells showed pairing-specific enhancement of the 5-HT-evoked depolarization (5 of 6 cells). In both types of experiments pairing usually enhanced the 5-HT-evoked increase in input resistance. Furthermore, pairing spike activity with bath application of 10^{-4} M IBMX enhanced both the depolarizing response and the increase in input resistance (4 of 5 cells). Pairing never reduced the depolarization or change in input resistance produced by the modulatory stimulus. These results suggest that the pairing-specific slow depolarization may involve both 5-HT and cAMP.

Pairing spike activity with sensitizing cutaneous stimulation (tail shock) transforms a hyperpolarizing response into a slow depolarization. This change in potential might contribute to the associative process by enhancing a tonic influx of Ca^{++} during the time that cyclic AMP activity is increased by the sensitizing stimulus. We are now examining whether voltage-dependent Ca^{++} currents can be modulated by the observed changes in membrane potential. An implication of this model is that lateral inhibition functions to sharpen the contrast between the associative and nonassociative effects of modulatory signals by reducing Ca^{++} influx in inactive cells.

107.1 PHOTOAFFINITY LABELLING OF ENKEPHALIN RECEPTOR OF RAT BRAIN PLASMA MEMBRANE. C.W.T. Yeung* (Spon.: W.G. Tatton). Playfair Neuroscience Unit, University of Toronto, M5T 2S8 Canada.

A photoreactive derivative of enkephalin, N^{α}-p-azidobenzoyl-Tyr^1-D-Ala^2,Met^5-enkephalin (AZB-Enk), was synthesized by reacting [D-Ala^2,Met^5]-enkephalin with equimolar of p-azidobenzoyl N-hydroxysuccinimide ester in dimethylforamide in the presence of triethylamine for 48 hours followed by gel filtration on Biogel P-2 eluted with 0.05M NH_4HCO_3 pH 8.4. The purity of AZB-Enk was assessed by thin layer chromatography on silica gel with 3 different solvent systems and found to have a single major spot with trace of unreacted enkephalin; n-butanol: acetic acid: water (4:1:1) R_f = 0.56; n-butanol: ethanol: 2N NH_4OH (5:1:2) R_f = 0.46; and carbon tetrachloride:methanol (95:5) R_f = 0. High voltage paper electrophoresis of AZB-Enk in pyridine acetate pH 6.4 at 4000 volts for 1 hour showed a single spot migrating at 4.1 cm from the origin towards the cathode. The photoreactivity of the analog was tested by its time dependent change in U.V. spectral properties on exposure to light. Competition of stereospecific binding of [^{3}H]-naloxone by AZB-Enk showed a K_D of 2.7 X 10^{-6}M, compared with a K_D of 1.75 x 10^{-8}M for [D-Ala^2,Met^5]-enkephalin. Radioactive AZB-Enk was prepared by iodination with [^{125}I]-iodine and chloramine-T. The tracer was purified by gel filtration and ion-exchange chromatography. [^{125}I]-AZB-Enk was photoreactive and had receptor binding activity. Rat brain plasma membrane was incubated with [^{125}I]-AZB-Enk and photolysed. SDS-gel electrophoresis of the solubilized and reduced membrane showed that a radioactive band with an apparent molecular weight of 38,000 daltons was specifically labelled.

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107.2 MUTANT NEUROBLASTOMA-GLIOMA CELLS SUPERSENSITIVE TO OPIATE ANTAGONISTS - A MODEL SYSTEM FOR OPIATE-TOLERANT STATE. R. Simantov, R. Levi* and H. Nadler*. Dept. of Genetics, Weizmann Inst. of Sci. Rehovot 76100, Israel.

A major obstacle in elucidating the mechanism of tolerance and dependence to opiate alkaloids in vivo is the involvement of multiple physiological pathways in the addicted state. Chronically treated animals, humans and cultured cells are supersensitive to opiate antagonists, presumably because the continuous presence of the opiate agonist induces biochemical compensatory systems that become hyperactive upon addition of the antagonist. In this study we have used the neuroblastoma-glioma NG108-15 hybrid cells to select two mutant clones (M-1 and M-2) that respond in the culture as if they were treated chronically with opiates. Forty eight hours treatment with the opiate antagonists naloxone and levallorphan increased the number of opiate receptors in neuroblastoma-glioma cells in stereospecific, time and dose dependent ways. Interestingly, naloxone had an enhanced effect on the density of receptors in the M-1 and M-2 clones as compared to the parent NG108-15. The mutant cells had also low content of calmodulin and their purified membranes showed decreased Ca^{++}-ATPase activity. Naloxone increased the basal and the Ca^{++} stimulated ATPase activity in all the clones tested but clones M-1 and M-2 were supersensitive as compared to the parent cells. The effect of other antagonists and opioid peptides was also studied. Modulation of Ca^{++}-ATPase activity by opiates via changes in calmodulin localization in the cells is suggested. The newly selected mutants M-1 and M-2 may serve as a model system to study the biochemical changes that control the enhanced response of opiate-addicted subjects to opiate antagonists and the interrelationship between the supersensitivity to antagonists and tolerance to opiate agonists.

107.3 EVIDENCE FOR σ-OPIOID RECEPTOR: BINDING OF PSYCHOTOMIMETIC OPIOID [^{3}H]-SKF-10047 (N-ALLYLNORMETAZOCINE) TO ETORPHINE-INACCESSIBLE SITES IN GUINEA-PIG BRAIN. T.-P. Su* (Spon: C. W. Gorodetzky). NIDA Addiction Research Center, Lexington, KY 40583.

Specific binding of the prototypic σ-opioid receptor agonist [^{3}H]-SKF-10047 (SKF) to guinea-pig brain homogenate was not completely inhibited by the strong narcotic analgesic l-etorphine. Properties of binding of [^{3}H]-SKF to these etorphine-inaccessible sites (EI sites) were examined. The specific binding of [^{3}H]-SKF to the EI sites is saturable. Scatchard analysis of the saturation curve revealed a single class of binding sites with apparent K_d of 252 nM and an estimated B_{max} of 663 f·mole/mg protein. The EI binding was reduced by heat treatment, trypsin digestion and phospholipase C digestion. The presence of Na^+ slightly increased specific EI binding. Lithium ion increased the EI binding by about 38% at the optimal concentration of 1 mM. Divalent cations such as Mg^{++}, Ca^{++} and Mn^{++} reduced EI binding. Ligand selectivities of the EI sites were completely different from those for the μ-type opiate receptor. Traditional μ-opiate receptor ligands such as morphine, naloxone and naltrexone were poor inhibitors (IC 50's > 25,000 nM), whereas opioid derivatives such as pentazocine, cyclazocine and SKF were potent inhibitors (IC 50's = 86, 102, 254 nM, respectively). The ligand selectivities of the EI sites also exhibited stereoselectivity which was the reverse of that for the μ-opiate receptor. Dextrorotatory isomers of pentazocine, cyclazocine and SKF were more potent than their levorotatory isomers. Similarly, dextrallorphan, dextromethorphan and dextrorphan were six to forty times more potent than their levo-isomers. Phencyclidine was about one-tenth as potent as SKF. Several other nonopioid drugs were among the most potent inhibitors. Examples are haloperidol, imipramine and propranolol (IC 50's = 7, 231, 627 nM, respectively). Analysis of EI binding data also indicated possible existence of subclasses of binding sites in that haloperidol did not completely inhibit EI binding. Highest levels of EI sites were found in brainstem, midbrain and cerebellum. Striatum and cortex contained lower levels of EI sites. It is concluded that EI sites likely represent σ-opioid receptors which have been proposed to mediate the psychotomimetic action of several opioids and other drugs (Martin et al., JPET 197:517-532, 1976). These EI sites appear to be different from other receptor systems and may not represent a single homogenous receptor population.

107.4 CHARACTERIZATION OF SMOOTH MICROSOMAL AND SYNAPTIC MEMBRANE OPIATE RECEPTORS. B.L. Roth and C.J. Coscia (SPON: M.B. Laskowski). Dept. of Biochemistry, St.Louis Univ. Sch. Med., St. Louis, MO 63104.

In continuing studies on smooth microsomal and synaptic membranes from rat forebrain (Roth et al., J. Biol. Chem. 256, 10117, 1981), we compared the binding properties of opiate receptors in these two discrete subcellular populations. Scatchard and Hill plots of [^{3}H]-naloxone binding were similar to crude membranes. In both membrane populations low and high affinity [^{3}H]-naloxone binding sites were present. The relative numbers of high and low affinity sites were quite similar for both synaptic membranes and smooth microsomes. The enrichment of binding sites in these fractions was due to an overall increase in the number and not the affinity of the receptors.

When [^{3}H]D-ala^2-D-leu^5-enkephalin was used as ligand, microsomes possessed 30-50% fewer sites than did synaptic membranes, while myelin-enriched fractions possessed almost no DADL binding sites. In competition binding experiments microsomal opiate receptors lack the sensitivity to Gpp(NH)p shown by synaptic and crude membrane preparations. In this respect microsomal opiate receptors resembled membranes that were experimentally GTP-uncoupled with N-ethylmaleimide (NEM). Agonist binding to microsomal and synaptic membrane opiate receptors was decreased by 100 mM NaCl. Microsomal receptors were more sensitive to Na^+ than synaptic membrane sites and like NEM-treated crude membranes were capable of differentiating agonist and antagonists in the presence of 100 mM NaCl. Washing the membranes with 100 mM NaCl enhanced microsomal δ opiate receptor affinity while synaptic membrane receptors were unaffected. $MnCl_2$ (50-100 μM) reversed the effects of 100 mM NaCl and 50 μM GTP on binding of the μ-specific agonist [^{3}H]-dihydromorphine, in both membrane populations.

In conclusion, we have demonstrated that both smooth microsome and synaptic membrane preparations contain equivalent enrichments of opiate antagonist binding sites. The microsomal receptors are unable to distinguish agonists from antagonists in the presence of Gpp(NH)p or GTP and are similar to NEM-treated membranes. Thus microsomal opiate receptors might represent a convenient source of GTP-uncoupled opiate receptors. Supported by NSF Grant BNS 8114947.

107.5 NIGRO-STRIATAL TERMINALS BEAR CONFORMATIONALLY MALLEABLE TYPE 1 OPIATE RECEPTORS WHILE INTRINSIC STRIATAL CELL BODIES BEAR CONFORMATIONALLY STATIC TYPE 2 OPIATE RECEPTORS. W.D. Bowen*, A. Pert and C.B. Pert (SPON: J. Rosenblatt). Neuroscience Branch, NIMH, Bethesda, MD 20205.

The prototype μ receptor ligand, dihydromorphine (DHM), and the prototype δ receptor ligand, [D-Ala^2,D-Leu^5]-enkephalin (D-ENK), bind to slide-mounted sections of rat striatum with distinctly different patterns. DHM binds discretely to patches (Type 1 pattern) while D-ENK binds diffusely (Type 2 pattern). However, we have found that allosteric effectors (Na+Mn+GTP) increase binding of D-ENK to patches and decrease binding of DHM without affecting D-ENK binding at diffuse sites. We have postulated that the Type 1 receptor is conformationally malleable with varying affinities for μ, δ, and κ ligands (Bowen et al. PNAS, 4818, 1981; Quirion et al. Adv. Endogenous and Exogenous Opioids, 63, 1981) while the Type 2 receptor is conformationally static and maintains a δ-like ligand selectivity (Olgiati et al. Life Sci., in press). Nigral 6-hydroxydopamine (6-OHDA) and striatal kainic acid (KA) lesions were used to ascertain the location of Type 1 and 2 opiate receptors on elements of striatum. Rats received unilateral injections of 6-OHDA (9 μg) into the substantia nigra or KA (1 μg) into the striatum and 4 and 6 weeks, respectively, were allowed to elapse before sacrifice of animals. Sections of striatum were prepared and incubated with ^{3}H-DHM and ^{3}H-D-ENK under conditions which label Type 1 receptors with highest affinity (Type 1 conditions): Mn for DHM and Na+Mn+GTP for D-ENK. Type 2 receptors were labeled by ^{3}H-D-ENK in presence of Mn + unlabeled naloxone (Type 2 condition), which minimizes binding to Type 1 receptors. (Concentrations are: NaCl, 100 mM; $Mn(OAc)_2$, 3 mM; naloxone, 2 nM; ^{3}H-DHM, 1 nM; ^{3}H- D-ENK, 2.5 nM). Sections were then subjected to autoradiography and the lesioned side compared to the unlesioned side by grain count analysis or computer optical density analysis. 6-OHDA caused approximately a 50% decrease in both DHM and D-ENK binding to Type 1 receptor patches. Type 2 labeling by D-ENK was not affected. By contrast, KA reduced D-ENK binding 70% under Type 2 conditions and 57% under Type 1 conditions. DHM was reduced only 36% by KA under Type 1 conditions. Thus, Type 1 receptors are sensitive to 6-OHDA lesioning while Type 2 receptors are not, indicating terminal localization. Type 2 receptors are more sensitive to KA lesioning than Type 1, indicating cell body localization. These results are consistent with the notion that Type 1 receptors are post-synaptic relative to opiatergic neurons which have Type 2 opiate receptors on their cell bodies. Furthermore, the observation that 6-OHDA lesioning decreases both μ and δ binding equally further supports the concept of a Type 1 receptor which can assume both μ-like and δ-like conformational states.

107.6 MODULATION OF AGONIST BINDING TO THE OPIATE RECEPTOR BY Na^+ AND GTP: EFFECT OF SULFHYDRYL REAGENTS. P. L. Kilian*, D. Mullikin-Kilpatrick*, and A. J. Blume. Roche Inst. of Molecular Biology, Nutley, N. J. 07110.

Binding of opioid agonist [^{3}H]D-ala^{2}met^{5}enkephalinamide (DAMA) to membrane-bound and CHAPS-solubilized opiate receptors from NG108-15 was measured. Receptors were assayed for binding of [^{3}H]DAMA in Tris/HCl buffer (pH=7.5) containing 4mM Tris-EDTA at 4°C for 2hr. Under these conditions, binding is at steady state and Scatchard analysis of saturation binding isotherms is linear. The membrane-bound and solubilized receptors have apparent Kds of ∿2 and ∿8nM, respectively. NaCl and GTP(Tris salt) were added 5min before the 2hr incubation with [^{3}H]DAMA. Na decreases agonist binding to the membrane-bound receptor (IC_{50} ∿30mM); this effect is specific for Na vs. other monovalent cations and appears to be due to a loss in agonist affinity. The potency of Na is increased two-fold by GTP or GTPγs. In contrast, the solubilized receptor is quite insensitive to Na (IC_{50} >100mM). Furthermore, this effect of Na is not very different from those accompanying increases in ionic strength. The sensitivity of the solubilized receptor to Na is increased by GTP or GTPγs (Na IC_{50} ∿ 30mM). The membrane-bound and solubilized receptors also differ in another respect. Decreases in [^{3}H]DAMA binding to the membrane-bound receptor induced by GTP or GTPγs require the presence of Na (GTP IC_{50} ∿ 1μM). Such a requirement for Na is not found for the solubilized receptor. The sensitivity of the solubilized receptor for GTP, however, is increased by Na (GTP IC_{50} minus Na ∿20μM; plus Na ∿ 5μM). The effects of 2mM NEM and 4mM DTT or diamide (added 10 min prior to [^{3}H]DAMA binding) were examined. In the absence of Na and GTP, these reagents have no significant effect on the number of membrane-bound receptors and, with the exception of NEM, no effect on the number of solubilized receptors. NEM decreases this latter value by two. The affinity of the membrane-bound receptor is decreased by diamide and NEM (app Kd changes from ∿2 to ∿4nM). In the presence of Na or GTP, these sulfhydryl reagents greatly alter [^{3}H]DAMA binding. The dose response curve for Na for the membrane-bound receptor is shifted to the left ∿2.5 fold by DTT or NEM while it is shifted to the right ∿3 fold by diamide. The inhibition by GTP of agonist binding to both the membrane-bound and solubilized opiate receptors is unchanged by DTT, but it is virtually eliminated by NEM or diamide. These findings suggest that a) alkylation by NEM or oxidation by diamide of a sulfhydryl group(s) results in loss of nucleotide sensitivity, b) sensitivity to Na is differentially altered by oxidizing vs. alkylating or reducing sulfhydryl reagents, c) the effects of Na and GTP on the opiate receptor are interrelated but can be distinghished by the action of sulfhydryl reagents and, d) sulfhydryl groups may play integral roles in opiate receptor function.

107.7 STEREOSPECIFIC DISPLACEMENT OF ^{3}H-PHENCYCLIDINE BINDING IN BRAIN MEMBRANES BY DEXTRORPHAN AND COMPARISON OF BEHAVIORAL EFFECTS IN DOGS. T. F. Murray, M. E. Leid*, B. J. Zaro*, and P. A. KLAVANO*. Colleges of Pharmacy and Veterinary Medicine, Washington State University, Pullman, WA 99164.

The findings that phencyclidine (PCP) discriminative stimulus properties in some species are shared with certain opoids such as cyclazocine, N-allylnormetazocine, and dextrorphan have suggested common neuronal substrates between PCP and some psychotomimetic opoids (Herling and Woods, Life Sci. 28:1571, 1981). The production of "PCP-like" stimulus properties appears to be mediated by an interaction with a stereospecific recognition site. Thus, while dextro (+)-isomers such as (+)-N-allymormetazocine, dexoxadrol and dextrorphan produce PCP-like discriminative stimulus effects, their corresponding levo-(-)isomers fail to produce PCP appropriate, responses. Our own investigations of the acute administration of PCP and ketamine to dogs indicated that i.v. doses of these dissociative anesthetics elicited a behavioral syndrome which was indistinguishable from the behavioral effects of dextrorphan and dextromethorphan in this species. Low i.v. doses of all four compounds produced excessive salivation and licking, dilated pupils, stereotyped head swaying and sniffing, with ataxia characterized by hypertonus of the hind limbs. Higher doses of all four compounds produced a loss of righting reflex which progressed to opisthothonus and tonic fore limb and clonic hind limb seizures. The rank order potency for these effects was PCP>ketamine>dextromethorphan>and dextrorphan. Based on these results we have developed a stereospecific receptor binding assay for ^{3}H-PCP recognition sites in rat brain membranes utilizing dextrorphan (300μM) to define nonspecific binding. The specific binding of ^{3}H-PCP (0.4-12nM) was examined in the presence and absence of dextrorphan while all assay tubes contained 0.1μM levorphanol to eliminate binding to μ receptors. Assays in which leucine-enkephalin (0.1μM) was added to all tubes along with levorphanol yielded identical results. We have employed both a filtration method using GF/C filters presoaked in 0.1% w/v polylysine, and a centrifugation procedure with a 3 min. centrifugation of samples at 15,000XG in a microfuge which attains maximum RPM in 3 seconds. Both procedures yielded identical results and a 60 minute incubation at 0-4°C was used in both cases. In competition assays specific binding percent inhibition was transformed to logit values and plotted against the logarithm of the displacer. Dextrorphan was found to displace ^{3}H-PCP with an IC_{50} of 1.3μM and a Hill Coefficient of 0.92, while levorphanol had no effect on the specific binding of ^{3}H-PCP at concentrations up to 300μM. Scatchard analysis of ^{3}H-PCP binding saturation isotherms indicated that ^{3}H-PCP had a K_D of 2.7nM in membranes prepared from the rat forebrain with a Bmax of 3.8p moles/g tissue. Characterization of PCP receptors is viable.

107.8 RELATIVE INVOLVEMENT OF μ AND δ OPIOID MECHANISMS IN MORPHINE-INDUCED DEPRESSION OF RESPIRATION IN RATS. Susan J. Ward[1] and John W. Holaday[2]. Department of Pharmacology, University of Minnesota, Minneapolis, Mn 55455[1] and Department of Medical Neurosciences, Walter Reed Army Institute, Washington DC 20012[2].

Although it is widely accepted that opioids depress respiration, the nature of the opioid receptor subtypes(s) that mediate these effects has not been elucidated. In the present study, male Sprague-Dawley rats were implanted with tail artery and jugular vein catheters, and right lateral ventricular head mounts 24 h prior to an experiment. Respiratory rate, pO_2 and pCO_2 blood levels were determined in groups of 5 concious rats[2] 15 min prior to, and 20, 40 and 60 min following, injection of morphine (i.v.). Peak changes in each of these parameters occurred 20 min following injection. Morphine (2, 4, 8 and 16 mg/kg i.v.) produced dose-related decreases in pO_2 levels and concomittant dose-related increases in pCO_2 levels in saline pretreated animals. Respiratory rate was not significantly altered by any of the doses of morphine used, and the within-group variation in respiratory rate changes was large. When animals were injected with a moderately selective δ antagonist ICI M 154,129, (200 nmol/rat i.c.v.) 5 min following administration of morphine (i.v.), the dose-response curves for morphine's effects on pO_2 and pCO_2 levels were shifted to the right 5.5- and 4.1-fold respectively. In animals pretreated with the highly selective non-equilibrium μ antagonist β-funaltrexamine (β-FNA)(5 nmol/rat i.c.v.) 18 h prior to testing, the dose-response curves for morphine's effects on pO_2 and pCO_2 levels were shifted to the right 6.1- and 4.4-fold respectively.[2] Using the radiant sheat tail flick test (with a cut-off time of 10 s) morphine, 4 mg/kg (i.v.), increased reaction times from 5.04 ± 0.77 to 9.41 ± 0.57 s in saline pretreated animals. In the presence of ICI M 154,129 morphine, 4 mg/kg (i.v.), had significantly less effect upon reaction (4.82 ± 0.88 to 6.99 ± 1.49 s). In β-FNA pretreated (5 nmol/rat i.c.v.) animals, morphine, 8 mg/kg (i.v.), had only a small effect upon reaction time (4.35 ± 0.21 to 6.21 ± 0.40 s). Neither β-FNA nor ICI M 154,129 given alone had a significant effect upon respiratory parameters or tail flick reaction times. Since both β-FNA and ICI M 154,129 antagonised the respiratory depressant actions of morphine, the results indicate that both μ and δ receptor populations appear to be involved in morphine-induced respiratory depression. The data would also suggest that respiratory rate is not a good indicator of morphine-induced depression of respiration in rats.

107.9 MORPHINE-INDUCED BRADYCARDIA IS PREDOMINANTLY MEDIATED AT MU SITES, WHEREAS MORPHINE-INDUCED HYPOTENSION MAY INVOLVE BOTH MU AND DELTA OPIOID RECEPTORS. John W. Holaday and Susan J. Ward*[1]. Neuropharmacology Branch, Dept. Med. Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012 and [1]Department of Pharmacology, University of Minnesota School of Medicine, Minneapolis, MN 55455.[2]

Activation of opioid receptors is known to result in a depression of cardiovascular function. This occurs either following opiate injection or endogenous opiate release (e.g., circulatory shock). An antagonist which blocks the adverse cardiovascular effects of opioids without blocking analgesia would have potential clinical utility. It was therefore of interest to characterize the opioid receptor subtype(s) which mediate bradycardia and/or hypotension following opiate challenge. The cardiovascular and antinociceptive effects of intravenous (iv) morphine sulfate (MS; a prototype mu agonist) were compared among rats treated with saline, β-funaltrexamine (βFNA; a long-lasting antagonist at mu sites), or ICI-M154,129 (ICI; an antagonist at delta sites). Male Sprague-Dawley rats (250-300 g) were surgically prepared with catheters in the external jugular vein and tail artery as well as an intracranial guide tube for right-lateral ventricular (icv) injections (20 µl over 20 sec). βFNA (5 nM) was administered icv 18 hrs. prior to MS challenge, whereas saline or ICI (0.2 µM) were injected icv 5 min after MS doses. Doses and treatment times for these opioid antagonists were determined from pilot studies. Cardiovascular and nociceptive responses following iv MS challenge were evaluated in unrestrained, conscious rats 24 hrs after surgery. Saline, βFNA, and ICI by themselves did not alter cardiovascular variables or nociceptive latencies at this time. Consistent with the hypothesis that opiate antinociception is predominantly mu mediated, βFNA pretreatment almost completely blocked the elevation of tail-flick latencies produced by MS (8 mg/kg iv), whereas ICI only partially reversed the antinociceptive effects of a lower dose of MS (4 mg/kg iv). However, βFNA pretreatment resulted in a 15-fold rightward shift of the bradycardic dose response to MS, whereas ICI injection produced only a 3.5-fold rightward shift. Both βFNA and ICI resulted in a 6-8 fold rightward shift of the hypotensive response to morphine challenge. Collectively, these data indicate that morphine bradycardia is predominantly mediated at mu sites, whereas the hypotensive effects of morphine may involve both mu and delta actions.

[2]We thank Drs. Takemori and Portoghese for the gift of βFNA and Dr. Turnbull of ICI Pharmaceuticals for the M154,129.

107.10 FUNCTIONAL ANALYSIS OF MYENTERIC δ RECEPTORS - A. R. GINTZLER* J.SCALISI* (SPON: J. RANCK). DEPT. BIOCHEMISTRY, DOWNSTATE MEDICAL CENTER, BROOKLYN, N.Y. 11203

Experiments were intiated in an attempt to obtain physiological data consistent with the presence of δ receptors in the guinea pig myenteric plexus and to determine if the release of enteric acetylcholine (reflected by 0.1 Hz electrically induced contractions) is under the control of this opiate receptor subtype. The basic scientific strategy employed was to determine whether ilea taken from guinea pigs chronically exposed to morphine manifested differential tolerance to the inhibitory effects of various opioid alkaloids (morphine and normorphine), and the opioid peptides DADLE & D-met^2-Pro^5-enkephalinamide (DMPE) on the magnitude of electrically induced contractions.

The results indicate that ilea taken from guinea pigs that had been chronically exposed to morphine exhibit a greater tolerance to morphine than to DADLE or D-met^2-Pro^5-enkephalin (DMPE). This strongly implies the existence of at least two different types of opiate receptor in the guinea pig myenteric plexus or two very different mechanisms of interaction between opioids and their receptor complex. Since acetylcholine is the transmitter mediating responses to 0.1 Hz stimulation these results would indicate that release of this transmitter is under the control of both µ & σ types of opioid receptor. Supported by NIDA GRANT Da 02893.

107.11 NALOXONAZINE: A POTENT LONG-ACTING INHIBITOR OF OPIATE BINDING SITES AND MORPHINE ANALGESIA. E. F. Hahn*and G. W. Pasternak. Laboratory of Biochemical Endocrinology, Rockefeller Univ., The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering, and Departments of Neurology and Pharmacology, Cornell U. Medical College, New York, N.Y. 10021

Naloxazone, the hydrazone derivative of naloxone, has proven useful in studies of opiate binding site heterogeneity both in vitro and in vivo based upon its long-acting inhibition of high affinity, or mu_1, binding sites. However, the need for high doses of naloxazone to inactivate the mu_1 sites raised the possibility that its actions might result from lower concentrations of a more active compound. We now present evidence that this more active compound is the azine derivative of naloxone: naloxonazine. In acidic solutions, approximately 35% of naloxazone spontaneously rearranges to its azine. Unlike naloxazone, naloxonazine is stable in solution. It does not appreciably dissociate into naloxone and naloxazone and no further conversion to other products can be detected. Naloxonazine seems to be responsible for the long-acting actions of naloxazone. Under assay conditions in which no azine is formed, naloxazone at concentrations up to 1000 nM does not inhibit opiate binding in a long-acting manner. Naloxonazine, on the other hand, inhibits the binding of both ^{3}H-dihydromorphine and ^{3}H-D-ala-D-leu-enkephalin over 90% at the same concentration (1000 nM). Extensive washing of the membranes after exposure to naloxonazine does not reverse this inhibition. Naloxonazine shows the same binding site selectivity as naloxazone. Exposure of tissue homogenates to low concentrations of naloxonazine (50 nM) followed by extensive washes, selectively inhibits the high affinity (mu_1) binding site, with partial inhibition seen at lower concentrations (10 nM). In vivo, naloxonazine blocks morphine analgesia for over 24 hours far more potently that naloxazone. In summary, the actions of naloxazone both in vivo and in vitro appear to be the result of its rearrangement to its azine derivative, naloxonazine.

107.12 CONFORMATIONALLY CONSTRAINED CYCLIC ENKEPHALIN ANALOGUES SHOW ENHANCED DELTA RECEPTOR SPECIFICITY. J.J. Galligan, H.I. Mosberg*, R. Hurst*, V.J. Hruby*, D.L. Kreulen and T.F. Burks. Depts. of Pharmacology and Chemistry*, University of Arizona, Tucson, Az. 85724.

There are several populations of opiate receptors which can be distinguished by the relative potencies of different agonists for binding to a particular receptor. We have synthesized and characterized the biological activities of four half-penicillamine containing enkephalin analogues which are highly specific δ receptor agonists. [D-Pen^2, D-Cys^5] enkephalinamide and enkephalin as well as [D-Pen^2, L-Cys^5] enkephalinamide and enkephalin were synthesized using solid phase methods and were purified by gel chromatography. Homogeneity was established by thin layer chromatography using three different solvent systems. Both enkephalinamides (1, 3.3 and 10 µg icv.) produced thermal analgesia (55.5 °C hot plate assay) in rats which lasted approximately 30 min. The free carboxy analogues produced a brief analgesia (10 min.) only at the highest dose (10 µg icv.). The enkephalinamides were equipotent in suppressing the electrically evoked contractions of the guinea pig ileum (GPI) while [D-Pen^2, D-Cys^5] enkephalin was the least potent agonist tested in this preparation. [D-Ala^2, D-Leu^5] enkephalin and [D-Pen^2, L-Cys^5] enkephalin were the most potent agonists in the mouse vas deferens (MVD) assay with both cyclic enkephalinamides showing enhanced potency in this preparation.

Agonist	IC_{50} (nM) GPI	MVD	GPI/MVD
Normorphine	91 ± 19	540 ± 113	.17
[D-Ala^2, D-Leu^5] enkephalin	24.3 ± 5.3	0.27 ± .06	90
[D-Ala^2, Met^5] enkephalinamide	2.2 ± 0.4	3.7 ± .04	.59
[D-Pen^2,D-Cys^5] enkephalinamide	117 ± 21.4	16.8 ± 3.1	6.9
[D-Pen^2, D-Cys^5] enkephalin	1347 ± 341	6.3 ± 1.2	214.8
[D-Pen^2, L-Cys^5] enkephalinamide	118 ± 18.6	3.6 ± .67	32.4
[D-Pen^2, L-Cys^5] enkephalin	212.6 ± 63	0.32 ± .03	664.4

The GPI is believed to contain largely µ opiate receptors while the MVD contains predominately δ receptors. Based on these assumptions the four cyclic analogues we have synthesized show a marked specificity for the δ receptor. This is particularly true for [D-Pen^2, L-Cys^5] enkephalin. Although this peptide is equipotent with [D-Ala^2, D-Leu^5] enkephalin in the MVD its relatively high IC_{50} in the GPI indicates a much greater specificity for the δ receptor. This is supported by the inability of the cyclic enkephalins to produce thermal analgesia. These compounds will be useful in studies of the conformational requirements for binding to a particular class of opiate receptor as well as determination of the physiological function of the δ opiate receptor. (Supported by USPHS grants DA02163, NS15420 and AM17420 and a PMAF predoctoral fellowship)

108.1 NEURAL ELEMENTS OF THE NUCLEUS MEDIANUS RAPHE AND INSTRUMENTAL BEHAVIOR. K.E. Asin and H.C. Fibiger. Neurological Sciences, Univ. British Columbia, Vancouver, B.C. Canada V6T 1W5

Following electrolytic lesions of the median nucleus of the raphe (MR), animals show a number of behavioral changes which are strikingly similar to those seen after damage to limbic structures (1,2). Since the paramedian tegmentum of the midbrain in the area of the MR is replete with serotonergic (5HT) and non-5HT cells and fibers of passage, the question arises as to the neuronal bases of the behaviors seen after electrolytic MR lesions. Last year we presented evidence that that open field hyperactivity and disrupted spontaneous alternation are likely due to damage to fibers of passage. In the current study, we examined the effects of various MR lesions on the acquisition and extinction of a bar press response and on the acquisition of an 8-arm radial maze task.

Subjects were male Wistar rats weighing about 300g and were randomly assigned to one of six treatment groups: An intra-MR injection of 5,7-dihydroxytryptamine (-DHT)(7.5ug/1.5ul) in ascorbate or vehicle alone; an intra-MR injection of ibotenate (6ug/1ul) in phosphate buffer or vehicle alone; an electrolytic MR lesion (1ma/8sec); anesthetization and "sham" operation. Three weeks after surgery rats were put on a 23h food deprivation schedule and CRF training was begun 8 days later and continued for 25 days. The response was then extinguished. Rats were then trained on a free running 8-arm maze task for 12 days. On the next three days rats were replaced into a start arm after entrance into any of the 7 remaining arms and were given 7 trials per day. This procedure was used to disrupt a position habit which some rats had acquired during the free running days. Rats were then sacrificed and their brains assayed.

Biochemical analysis indicated that all lesioned rats showed about a 50% reduction in hippocampal and frontal cortex 5HT. An ANOVA on the lever pressing data indicated that none of the experimental groups differed from controls during the last 6 days of acquisition or during extinction. In contrast, analysis of the 8-arm maze data indicated that rats with electrolytic lesions showed more incorrect arm entries both on the last 4 days of free-running training and on the replacement task; none of the other groups differed from controls.

These results suggest that although rats with electrolytic MR lesions are impaired on the acquisition and extinction of a runway task (1) they are not so impaired on a CRF lever press task. Furthermore, it appears that their impaired 8-arm maze performance (3) is due to the destruction of fibers of passage.

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2. -ibid- Behav. Neur. Biol. 28, 408, 1980.
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108.2 EVIDENCE THAT SOME OF THE BEHAVIORAL EFFECTS OF LSD ARE MEDIATED BY A DIRECT ACTION AT POSTSYNAPTIC SEROTONERGIC RECEPTORS. Barry L. Jacobs, Kurt Rasmussen, and James Heym. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

We have recently reported that there are a number of important dissociations between the behavioral effects of hallucinogenic drugs and their effects upon serotonergic unit activity (Science 205:515-518, 1979; Brain Res. 215:275-293, 1981). Because of this we have begun to refocus our attention on the direct postsynaptic serotonergic effects of LSD and related hallucinogens. When cats were administered mianserin (0.1, 0.25, or 1.0 mg/kg i.p.), a 5HT antagonist, 30 min before LSD (50 µg/kg i.p.), it blocked LSD's characteristic behavioral effects in a dose-dependent manner. This is probably attributable to a serotonergic action since cyproheptadine had a similar effect. This blockade did not appear to be due to non-specific effects, since administration of mianserin alone produced no decrease in spontaneous activity and the animals displayed no signs of sedation or catalepsy. Furthermore, mianserin exerted no blocking action on the behavioral effects of high doses (4 mg/kg) of the dopamine agonist apomorphine, but did block the behavioral effects of the hallucinogen 2,5-dimethoxy-4-methamphetamine (DOM). We then attempted to determine whether this blocking action of mianserin was due to a pre- or postsynaptic effect. When we pretreated animals with mianserin (1 mg/kg) and then administered LSD (50 µg/kg) 30 min later, we observed, as above, an almost complete blockade of the behavioral effects of LSD, but no alteration of the 40-60% depression of serotonergic unit activity that LSD typically produces in neurons in nucleus raphe dorsalis and centralis superior. A related study provides evidence that LSD may be acting at a $5HT_1$ receptor. Chronic administration of nialamide (5 mg/kg i.p. once daily for a week) completely blocked the behavioral effects of both LSD (50 µg/kg) and DOM (250 µg/kg), but had no influence on the behavioral effects of apomorphine (4 mg/kg). By contrast, administration of amitriptyline (5 mg/kg i.p. once daily for a week) had no blocking action on LSD's behavioral effects. Previous receptor binding studies in rats indicate that chronic administration of both nialamide and amitriptyline decreases the availability of $5HT_2$ receptors, but that only nialamide decreases $5HT_1$ receptor binding. In conclusion, these data emphasize the behavioral importance of the postsynaptic serotonergic effects of LSD. (Supported by MH 23433).

108.3 CHLORAL HYDRATE ANESTHESIA ALTERS THE RESPONSIVENESS OF SEROTONERGIC NEURONS WITHIN THE DORSAL RAPHE NUCLEUS. James Heym, George F. Steinfels and Barry L. Jacobs, Prog. in Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

We have previously reported that serotonergic neurons within the dorsal raphe nucleus (DRN) of freely moving cats can be driven by phasic auditory or visual stimuli (Brain Res. 232: 29-40, 1982) and are relatively unaffected by systemic administration of drugs that decrease adrenergic neurotransmission (Eur. J. Pharmacol. 74: 117-125, 1981). These findings are in contrast to studies in chloral hydrate anesthetized rats where the discharge rate of serotonergic neurons within the DRN has been reported to be unaffected by light flash (Mosko and Jacobs, Physiol. Behav. 13: 587-593, 1974), but heavily dependent upon an α adrenergic input (Baraban and Aghajanian, Neuropharmacology 19: 355-363, 1980). The basis for these conflicting results might be attributable to the difference between experimental preparations, i.e. anesthetized versus freely moving subjects. In an attempt to clarify this issue, we compared the effects of sensory stimulation or adrenergic blockade on DRN serotonergic unit activity in chloral hydrate anesthetized cats with those observed in freely moving cats. Animals were prepared for chronic unit recording using a technique previously described in detail (Brain Res. 163: 135-150, 1979). After recording pre-drug baseline activity and unit responses to sensory stimulation, cats were administered chloral hydrate (250-300 mg/kg, i.p.) to obtain a level of anesthesia in which there was an absence of a withdrawal response to paw pressure. While in this anesthetized condition, the discharge of DRN serotonergic neurons was more regular and approximately 20% slower than in a quiet awake animal. However, in these anesthetized cats, we found the firing rates of serotonergic cells to be unresponsive to either phasic auditory or visual stimuli. Additionally, the discharge of these neurons was rapidly and completely suppressed after systemic injection of the α$_1$ adrenergic receptor antagonist WB4101 (1.0 mg/kg, i.p.). Thus, these results are contrary to our findings with freely moving cats, but are in accord with those previously reported for chloral hydrate anesthetized rats. These data may explain some of the discrepancies in results between electrophysiological studies conducted in chloral hydrate anesthetized animals and those utilizing freely moving subjects. Furthermore, the present study indicates that chloral hydrate anesthesia, while having relatively small effects on spontaneous activity, may profoundly alter the responsiveness of DRN serotonergic neurons to afferent inputs and pharmacological manipulations. (Supported by MH 23433 and NS 06269)

108.4 DISCHARGE CHARACTERISTICS OF DORSAL RAPHE (DRN) NEURONS DURING REPEATED SLEEP-WAKE CYCLES. R. Lydic, R.W. McCarley and J.A. Hobson. Lab. of Neurophysiol., Harvard Medical School, Boston, MA

The ultradian alternation between synchronized (S) and desynchronized (D) sleep has been hypothesized to be produced by oscillations in the activity of cholinergic neurons from the median pontine reticular formation (mPRF) (D-on cells) and by aminergic neurons in the locus coeruleus and midbrain raphe nuclei (D-off cells). Using microwire recording electrodes similar to those of Jacobs, we have undertaken long-term recordings and time-normalized sleep cycle averaging of D-off cell discharge rates. These data were used to characterize the continuous time course of cellular activity with respect to sleep cycle phase and goodness to fit with the mathematical predictions of the reciprocal interaction model. Data were obtained from extracellular recordings of four slowly discharging D-off cells using microwires (32 and 62 micron diameter) aimed at the DRN; recordings lasted for 4 hrs and for 3,6, and 13 days and included over 200 complete sleep cycles.

The time course and shape of D-off cell activity curves and the period length of the sleep cycle (D end to D end) were functions of the amount of prior behavioral activity. Cats with 12 to 16 hrs of prior activity had shorter, more regular cycles with the following time course of cellular discharge: Neuronal activity peaked in the initial portion of the cycle associated with waking (W). An abrupt decline in cell activity began about the 4th decile associated with the onset of PGO waves. The lowest neuronal discharge level was observed at the initial segment of the D-period which also contained the maximum number of PGO waves. A small increase in D-off cell activity occurred near the end of the D-period. The time course of cellular activity paralleled the theoretical curve for D-off cells predicted by the Lotka-Volterra equations of the reciprocal interaction model. In the absence of extended periods of prior behavioral activity, cats had longer, more irregular sleep cycles with longer periods of W and S. State dependent D-off cell excitability changes, inferred from mPRF stimulation, paralleled the spontaneous discharge curves.

Sleep state and the time course of D-off cell discharge are predictably modified by behavioral activity which precedes recordings from either head restrained or unrestrained cat. The time course of D-off cell activity is compatible with their playing an active role in the physiological events of waking and a permissive, disinhibitory role in generating D phenomena.

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108.5 EFFECTS OF SENSORY STIMULATION ON DOPAMINERGIC UNIT ACTIVITY IN FREELY MOVING CATS. George F. Steinfels, James Heym, Robert E. Strecker and Barry L. Jacobs. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

In a previous study we reported on the unit activity of dopaminergic (DA) neurons in the substantia nigra (SN) of awake, freely moving cats (Life Sci. 29:1435, 1981). Although dopamine has been postulated to play a role in sleep-waking behavior, we reported that the discharge rate and pattern of these neurons remained constant across this cycle. We concluded that, at the cellular level, an involvement of SN DA neurons in the sleep-waking cycle was not apparent. Another hypothesis for the role of mesencephalic DA neurons in behavior has been in the central integration of sensorimotor information. We have continued our studies of DA neuronal activity in freely moving cats by investigating the response of these neurons to auditory or visual stimuli. Unit activity was recorded by means of a movable microwire technique (Life Sci. 29:1435, 1981). During quiet waking, a click or light flash was presented once every 2 sec for a total of 64 trials. The predominant response to these stimuli was excitation (latency ≈ 50-70 msec/duration ≈ 60-90 msec) followed by inhibition (duration ≈ 80-150 msec) with no evidence of habituation. In a second series of experiments the clicks were presented continuously in order to assess the response of these neurons across the sleep-wake cycle. The initial excitatory response to the click which occurred during quiet waking diminished as the cat progressed into slow wave sleep and in some instances was virtually absent during REM sleep. Upon awakening from REM sleep, DA neurons once again displayed an excitatory response to the click. A final series of experiments examined the response of DA neurons to stimuli which elicited a behavioral orienting response in a cat. The change we observed was a rate decrease in association with orienting responses. The suppression was, however, sometimes preceeded by an initial short burst of unit activity. This type of effect was seen in over 50% of the cells in which we examined this relationship. The duration of the suppression ranged from 1-10 sec. and the mean discharge rate during the suppression was 63% below quiet waking baseline. As the orientation to these stimuli habituated, so did the associated DA unit suppression. Our study demonstrates that DA neurons respond similarly to auditory and visual stimuli which may indicate that the neural mechanism mediating this response may be common to both sensory systems. However, during sleep states the influence of this input upon the DA system is greatly attenuated. Thus, even though DA unit activity is stable across the sleep-waking cycle, the response to of these cells to environmental stimuli varies dramatically across behavioral states. (Supported by DA 05224-01, NSF BNS 81-19840, American Philosophical Society).

108.6 BEHAVIORAL ORGANIZATION OF THE A8 DOPAMINE CELL GROUP. A. Y. Deutch. Dept. of Psychology, Univ. of Georgia, Athens, GA 30602.

The A8 dopamine (DA) cell group has generally been considered to represent a caudal extension of the A9 (substantia nigra) DA neurons, and to contribute to the nigrostriatal system. However, recent data indicate that the A8 and A10 DA cell groups share common basal forebrain projection fields (Lenard and Nauta, Neurosci. Let., Suppl. 6: S52, 1980). In light of these anatomical findings, the behavioral consequences of A8 lesions were assessed in order to characterize the behavioral organization of this nuclear group.

Unilateral and bilateral 6-hydroxydopamine lesions of the A8, A9, or A10 cell groups were placed in adult male rats pretreated with DMI. The animals were subsequently tested for alterations in behaviors previously reported to occur as a consequence of either A9 or A10 lesions: rotational behavior, contralateral sensory neglect, amphetamine (AMP)-induced stereotypy, and locomotor activity. Lesion extent was determined by flourescent histochemistry.

Unilateral A8 lesions resulted in very weak AMP-induced rotational behavior, which differed from that observed in sham-lesioned subjects only in directional (ipsilateral) bias, but not magnitude. A9 lesions effected very high rotation rates. Contralateral sensory neglect was not observed in A8-lesioned subjects, in distinction to the mild inattention to contralaterally presented stimuli in animals with A9 lesions.

Bilateral lesions of the A8 cell group appeared to minimally attenuate the development of stereotyped behavior patterns secondary to high (5 mg/kg, ip), but not low or moderate (1 or 2 mg/kg) doses of AMP; biting and licking behaviors were weakly reduced in A8-lesioned subjects. Bilateral A8 lesions resulted in a significant increase in spontaneous locomotor activity, similar in both magnitude and temporal organization to the hyperactivity observed following A10 lesions. The A8 lesions also altered the time course of AMP-induced activity in that the duration was shortened.

These data therefore suggest that the behavioral organization of the A8 cell group more closely resembles that of the A10 (mesolimbic) rather than the A9 (nigrostriatal) system. The A8 cell group may be critically involved in the modulation of mesolimbic activity, and further modulate nigrostriatal function both directly and indirectly via mesolimbic alterations.

108.7 BEHAVIORALLY INDUCED DIFFERENTIAL DOPAMINE TURNOVER IN MESOTELENCEPHALIC PROJECTIONS. J.D. Miller, B.A. McMillen, S.G. Speciale, & D.C. German. Depts. of Physiol., Pharmacol., and Psychiat., U. of Texas Health Sci. Cntr, Dallas, TX. 75235.

The projection pathways of mesencephalic dopamine (DA) neurons have been subdivided into nigrostriatal, mesolimbic and mesocortical components. Previous work has shown that foot shock elevates DA metabolism in the mesocortical (medial frontal cortex) and mesolimbic (nucleus accumbens) components without eliciting any change in DA metabolism in the striatum. Conversely, certain behavioral paradigms (e.g. fixed ratio and variable interval operant schedules) elevate striatal DA metabolism without affecting the mesocortical projection. Presumably, the striatal enhancement reflects the vigorous motoric activity entailed by the behavioral task. This study was performed in order to examine the nigrostriatal projection, the mesocortical projection and the mesolimbic projection in a task that was specifically designed to be motor activating (wheel locomotion). For comparison, we examined the effects of foot shock stress on the same terminal regions.

Two groups of six female albino rats (Sprague-Dawley, 250-300 g) were either placed in a 0.5 m diameter wheel which rotated at 2 rpm for 1 hr or exposed to foot shock stress for 20 min (.8 mA, six 160 msec shocks/10 sec). A third group served as control. Subjects were immediately removed from the apparatus, and decapitated; their brains removed and placed in cold saline. The terminal regions mentioned above were dissected out by a micropunch procedure. DA, DOPAC, and the internal standard were separated from tissue by batch alumina extraction followed by HPLC with ECD. Turnover scores (DOPAC/DA) were calculated and are presented below (* = significantly differs from control).

	Control	Shock	Locomotion	p Value
Caudate	.082 ± .006	.110 ± .004*	.106 ± .001*	< .001
Front. Ctx.	.327 ± .049	.614 ± .089*	.289 ± .018	< .01
N. Accumbens	.191 ± .017	.205 ± .025	.169 ± .016	ns
Olf. Tubercle	.345 ± .021	.384 ± .020	.355 ± .016	ns

Analysis of variance indicated that DA levels were not altered by the behavioral tasks. Striatal turnover was elevated by both the locomotor task and foot shock, whereas the frontal cortex only showed an elevation in turnover in the foot shock paradigm. In the mesolimbic regions, no change in turnover was observed. The results indicate that DA neuronal activity in separate DA terminal regions may vary as a function of behavioral task parameters, e.g. relative involvement of motoric vs. affective factors.

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108.8 CHOLINERGIC AND CATECHOLAMINERGIC MECHANISM IN THE MONKEY ORBITOFRONTAL CORTEX NEURONS DURING FEEDING BEHAVIOR. Y. Oomura, H. Nishino*, T. Ono, S, Aou*, M. Inoue*, K. Yamabe*, S. K. Sikdar*, M. Hynes*, T. Katafuchi*, Y. Mizuno* and A. Inokuchi*. National Inst. of Physiol. Sci., Okazaki 444, Kyushu Univ., Fukuoka 812 and Toyama Med. & Pharm. Univ., Toyama 930-01, Japan.

To elucidate prefrontal neuronal mechanisms involved in motivated behavior and the role of subcortical cholinergic and catecholaminergic systems, effects of iontophoretic application of acetylcholine (ACh), noradrenalin (NA) and dopamine (DA) on the single unit activity of the rhesus monkey orbitofrontal cortex neurons during a high fixed ratio (FR 30) bar press feeding task, were investigated.

The 227 neurons studied, were assorted into 3 major types, based on their response patterns during the bar press period. a) Type D-BP: neurons showing decrease in activity during the bar press period (N=95, 42%); b) Type E-BP: neurons showing increase in activity during the bar press period (N=66, 29%); c) Type O-BP: neurons not showing any change in activity during the bar press period (N=66.29%). One-fourth of either Type D-BP or Type E-BP neurons increased in firing rate on application of ACh ($P<0.01$) and the ACh effects were blocked by iontophoretic application of atropine. The action of ACh was thus excitatory and nonspecific. It had no effect on Type O-BP neurons.

Seventy-five percent of Type D-BP neurons were suppressed by NA ($P<0.01$), while only 20% of the Type E-BP neurons were suppressed by NA. Noradrenaline did not have facilitatory effect on any of the neurons tested. Iontophoretic application of a ß-adrenoceptive blocker suppressed not only the NA effect but also the Type D-BP responses. Further NA was without effect on Type O-BP neurons.

Forty percent of Type E-BP neurons were excited by DA ($P<0.01$). Spiroperidol, a DA blocker suppressed not only the facilitatory effect of DA, but also the Type E-BP response. Again as with ACh and NA, DA insensitive cells were the Type O-BP neurons.

The importance of the catecholaminergic system in the neuronal regulation of motivation related feeding behavior is suggested by the present study, the noradrenergic and the dopaminergic systems exerting different functional effects. The cholinergic system on the other hand seems to exert a nonspecific control on the neurons related to operant feeding behavior.

108.9 LOCUS COERULEUS COMPLEX: CHRONIC UNIT RECORDING IN THE CAT WITH SPECIAL REFERENCE TO THE ATONIA OF PARADOXICAL SLEEP. P.B. Reiner and A.R. Morrison, School of Vet. Med., Univ. of Pa., Phila., Pa. 19104

The Locus Coeruleus Complex (LCx) has been implicated in the modulation of a wide variety of neural and physiological functions, including the atonia of paradoxical sleep (PS). A subpopulation of these neurons has been termed 'PS-off' cells because their firing rates reduce dramatically during PS. Because dorsal raphe PS-off cells are more related to muscle tone than state (Trulson, et al., Brain Res., 226: 75,1981), we are testing the covariation of LCx discharge and muscle tone. As a first step, we have quantified the relationship of resumption of firing of PS-off cells and muscle tone at the end of PS.

52 neurons were recorded from the LCx of 3 cats using a chronic microwire recording technique which permitted free movement during recording sessions. Standard electrodes for monitoring sleep states were also implanted. 14 units (27%) in 3 cats which fired most in waking, less in slow wave sleep and virtually ceased firing in PS, were classified as PS-off cells. 7/14 were completely silent during PS. Mean firing rates/sec for active waking, quiet waking, slow wave sleep and PS were 2.54, 1.32, 0.32, and 0.06, respectively. In 10 units we examined the temporal relationship of the first detectable increase in EMG tone to the resumption of discharge in PS-off cells at PS termination. The first spike at PS offset was considered as time zero and the EMG change computed in relation to it. Marked variation was noted in the data (mean= -248msec) which ranged from -6800msec to +2000msec. More significantly, for units in which multiple samples were available (N=5), this temporal relationship remained highly variable, with resumption of unit activity occurring both prior to and after EMG onset for different episodes of PS by the same neuron, indicating the variability did not depend upon the muscles sampled by our EMG.

The data indicates that LCx PS-off neurons do not vary their discharge in tight synchrony with muscle tone. Presently, we hope to extend these results by examining the discharge of LCx neurons in cats with PS without atonia.

(Supported by NIH grants GM-07170 and NS-13110)

108.10 DIMINISHED IMPROVEMENT IN ACQUISITION OF A SPECIFIC LOCOMOTOR TASK AFTER LOCALIZED 6-HYDROXYDOPAMINE INDUCED CEREBELLAR NOREPINEPHRINE DEPLETION. Mark Watson and James G. McElligott (SPON: John J. O'Neill). Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

A newly developed rod runway paradigm was used to obtain evidence in support of the hypothesis that cerebellar norepinephrine (NE) is involved in motor learning. Previous work (Watson and McElligott, Soc. Neurosci. Abstr., 5:1192, 1979; Watson and McElligott, Fed. Proc., 40:241, 1981) has established that running times (RT, 25 trials/day, 4 consecutive days) of water-deprived rats tested on an equally spaced regular rod arrangement (REG), before and after intracisternal 6-hydroxydopamine (6-OHDA, 3x, 25ug/25ul free base) infusion (REG/REG), or on a more difficult unequally spaced irregular rod arrangement (IRR/IRR) showed no significant differences when compared to ascorbate vehicle controls. However, rats tested on the REG task before, and the new IRR task after similar 6-OHDA lesioning (REG/IRR), demonstrated a significant impairment in performance over the 4 day post-infusion period. To verify that depleted cerebellar NE was responsible for this observed deficit, a further study employed this same paradigm (REG/IRR) in combination with a localized lesion of the coeruleo-cerebellar pathway. This bilateral 6-OHDA (8ug/2ul) lesioning of the coeruleo-cerebellar pathway produced a similar, significant impairment in post-infusion RT. These rats however, showed no significant differences from vehicle controls upon concurrent testing of intertrial interval times in the runway, or in open field behavior or the reversal of a simple T-maze position habit. Thus, both the intracisternal (REG/IRR) and coeruleo-cerebellar (REG/IRR) lesioned rats, with cerebellar NE reduced by 85% and 75% respectively, showed impairment of acquisitional performance when presented with the new locomotor task (IRR) after 6-OHDA infusion. Moreover, the subsequent degree of cerebellar noradrenergic deafferentation was found to correlate with the degree of impaired acquisitional, but not with the post-acquisitional performance of the locomotor behavior. As a site of NE-related adaptive plasticity, increases in cerebellar NE may serve to increase capacity for motor learning.

108.11 DECREASED TRACE AMINE AND TRACE ACID LEVELS AFTER FIGHTING IN ISOLATED AGGRESSIVE MICE. C.T. Dourish*, B.A. Davis*, L.E. Dyck*, R.S.G. Jones* and A.A. Boulton. Psychiatric Res. Div., Univ. Hospital, Saskatoon, Saskatchewan S7N OXO, Canada.

Recently, Sandler et al. (Lancet ii, 1269, 1978) have claimed that phenylacetic acid (PAA), the major metabolite of β-phenylethylamine (PEA), was present in abnormally high levels in the plasma of violent prisoners compared to non-violent control prisoners. We have used a standard animal model of aggression, the isolation syndrome in mice, to examine the possible role of PEA and other related trace amines [para-tyramine (p-TA) and meta-tyramine (m-TA)] in aggressive behavior. Sixty male Swiss mice were randomly allocated to isolation (individually housed for 4 weeks) or group (housed in groups of 10 for 4 weeks) treatments. Groups of 3 mice previously isolated or group housed, respectively, were tested in metabolic cages. Aggressive behavior was rated on a standard scale. Twenty-four hours later, a urine sample, a blood sample, and whole brains, from each group were collected for analysis. The concentrations of PEA, PAA, m-TA, m-HPA (meta-hydroxyphenylacetic acid, the major metabolite of m-TA), p-TA and p-HPA (para-hydroxyphenylacetic acid, the major metabolite of p-TA) were determined by a gas chromatographic-mass spectrometric method. Isolated mice were hyperactive, hyperreactive and aggressive compared to group-housed controls, thus confirming many previous findings. The plasma levels of PAA and p-HPA and the urinary levels of PEA, PAA, m-TA, m-HPA and p-HPA were significantly decreased in the isolated aggressive mice [to 60% or less of the levels in group-housed controls ($p < .01$ in all cases, independent 2-tailed t-test)]. The brain concentrations of PEA, PAA, p-TA and p-HPA tended to be reduced in isolated animals although these effects did not achieve statistical significance. In contrast, the brain levels of m-TA and m-HPA and the plasma levels of m-TA, were slightly (but not significantly) increased in isolated mice. These data are at variance with those of Sandler et al. who reported an increase in plasma levels of PAA in aggressive prisoners, whereas we observed a decrease in plasma levels of PAA and p-HPA and urinary levels of PEA, PAA, m-TA, m-HPA and p-HPA in aggressive mice. It is interesting to note, however, that in a recent study in our laboratory (unpublished observations) Boulton et al. failed to replicate Sandler's findings. Boulton et al. found a slight, but not significant, increase in plasma PAA levels in violent offenders, but a reduction in plasma levels of p-HPA. Supported by Sask. Health and the M.R.C. of Canada.

108.12 RECEPTOR CHANGES in LEARNED HELPLESSNESS, J. Johnson*[1], A. Sherman[1], F. Petty[1], D. Taylor[2] and F. Henn[3]. [1]Dept. of Psychiatry, U. of Iowa, Iowa City, IA. 52242. [2]CNS Research, Bristol-Myers Co., Evansville IN. 47721. [3]Dept. of Psychiatry, State University of New York-Stony Brook, Stony Brook, L.I. N.Y. 11794.

Learned Helplessness$^{(LH)}$ is an animal model of depression which responds to numerous pharmacological interventions which are effective in clinical depression. In order to evaluate the CNS response to LH training α,β,GABA,LSD, diazepam and imipramine receptors were examined before and after training sufficient to cause behavioral changes. Only β receptors and imipramine receptors showed alterations and these were only seen in specific anatomical regions. β receptor changes were confined to the hippocampus and imipramine receptors were found altered in the anterior neocortex. In both cases the receptor changes were seen only in animals that expressed altered behavior, animals receiving a similar training schedule that did not show LH also did not show receptor changes. The Bmax for hippocampal β receptor went from 589 fmol/mg protein in controls to 875 fmol/mg protein in LH animals. The reverse was seen for imipramine binding in which controls had a Bmax in anterior neocortex of 794 fmol/mg protein while helpless animals went down to 586 fmol/mg protein. In order to see which of these responses might be primary the LH behavior was reversed with a variety of antidepressants. Tricyclic drugs decreased β receptors. However, allowing the animals to stay without treatment until the LH behavior reversed (over 2 weeks) resulted in animals without behavioral changes but with still increased β receptors. Thus it is possible to dissociate the β receptor increase and LH behavior. In order to look at the role of the imipramine receptor the novel antidepressant MH 13754 was examined. This drug did not alter β receptors and does appear to act at the 5 HT_2 site. It was found to reverse LH with an ED_{50} 50 mg/kg. Thus a compound with no α,β, or cholinergic activity is effective in reversing learned helplessness.

108.13 YOHOMBINE INDUCED ANXIETY AND INCREASED NORADRENERGIC FUNCTION IN HUMANS: EFFECTS OF DIAZEPAM AND CLONIDINE. D.S. Charney and G.R. Heninger, Dept. Psychiatry, Yale Univ. Sch. of Med., New Haven, Ct.

Several lines of research support the hypothesis that overactivity of brain noradrenergic (NA) systems are involved in the development of anxiety and the mechanism of action of antianxiety drugs. Pharmacological activation of brain NA neurons produces physiological and behavioral effects in monkeys and humans resembling naturally occurring anxiety states. Yohimbine, an alpha-2 adrenergic autoreceptor antagonist, produces mild anxiety and increases in plasma 3-methoxy-4 hydroxyphenylethyleneglycol (MHPG) and autonomic symptoms in humans. To assess the antianxiety mechanism of action of diazepam and the alpha-2 adrenergic autoreceptor agonist, clonidine, these effects of yohimbine were studied following diazepam and clonidine pretreatment.

Methods: Nine healthy subjects had six test days in random order: placebo; yohimbine (30 mg); clonidine (5 ug/kg); diazepam (10 mg); clonidine and yohimbine; and diazepam and yohimbine. Plasma was obtained from blood samples drawn through an indwelling intravenous catheter, both before and at a variety of time points following drug administration. Plasma MHPG was determined by gas chromatography and mass spectrometry. Visual analogue scales and a brief subjective and somatic anxiety scale were used to evaluate changes in behavior and physical symptoms.

Results: Yohimbine increased plasma MHPG by approximately 45% ($p<.05$), clonidine decreased plasma MHPG 20% ($p<.05$), and diazepam had no effect on plasma MHPG levels. Clonidine pretreatment significantly attenuated the yohimbine induced increase in plasma MHPG ($p<.05$), whereas diazepam did not. Yohimbine induced significant increases in subject rated anxiety ($p<.05$). In contrast to effects on plasma MHPG, both clonidine and diazepam antagonized the anxiety inducing actions of yohimbine ($p<.05$). Yohimbine produced autonomic changes such as increased blood pressure, rhinorrhea and piloerection. Clonidine but not diazepam reversed most of the yohimbine induced autonomic changes.

Summary: The attenuation of the yohimbine induced increases in MHPG, anxiety, and autonomic function by clonidine is supportive of the hypothesis relating increased NA function to anxiety states. Diazepam did not alter MHPG levels or the yohimbine induced increase in MHPG but did antagonize yohimbine induced anxiety. This indicates that the antianxiety action of diazepam may not be related to effects on NA function.

109.1 DOSE-DURATION STUDIES WITH DOPAMINERGIC (DA) AGONISTS AND ANTAGONISTS: ALTERATION IN BEHAVIORAL SENSITIVITY. P.M.CARVEY,* W.J. WEINER,C.GOETZ,*C.TANNER,* AND H.L.KLAWANS* (SPON: M. COHEN). DEPT. NEUROSCIENCES,RUSH-PRESBYTERIAN ST. LUKES MED. CENTER, CHICAGO,IL 60612.

We have studied the chronic effects of varying doses of Chlorpromazine (CPZ),Trifluoperazine (TRI,a rigid Clozapine analog (CLOZ),Haloperidol (HAL),Prochlorperazine (PRO),Thioridazine (THIO), Levodopa (DOPA),Lisuride (LIS),and Bromocriptine (BCT) on the stereotypic behavioral (SB) response to a fixed challenging dose of apomorphine HCl subsequent to treatment withdrawal. The SB response was increased following the withdrawal of CPZ,TRI,PRO, CLOZ, and HAL. The degree of behavioral supersensitivity (SS) to these agents was dependent upon the chronic treatment dosage utilized. The higher the chronic dosage, the greater the behavioral SS. THIO did not induce significant SS at doses up to 5 mg/kg. The conversion of the delivered chronic dosages into CPZ equivalents yielded dose-response curves which indicated that the risk of development of subsequent SS was dependent upon the relative anticholinergic activity of these agents. The greater the anticholinergic activity of an agent, the less likely it would be to produce SS at CPZ equivalent dosages.

DOPA,LIS, and BCT produced behavioral subsensitivity (SubS) to a fixed dose of apomorphine challenge following chronic treatment withdrawal. The degree of SubS also tended to be dose-dependent. The SubS SB response to apomorphine was present while the behavioral response to DOPA and LIS, but not BCT, was SS, indicating that reverse-tolerance can exist in the presence of apomorphine SubS.

The duration of varying doses of CPZ,HAL,LIS, and DOPA were also studied. Lower doses of CPZ and HAL, which were not SS at 3 weeks, produced significant SS, at some dosages, if the duration of treatment was extended to 6 or 9 weeks. Once SS was established to a dose of HAL, the degree of SS did not progress with time; this was not necessarily true however for CPZ. In an analagous fashion, once LIS and DOPA SubS was established, it did not continue to progress with time.

The results of these studies suggest that the CNS compensates around delivered loads of DA agents and that the degree of compensation is not only dependent upon the delivered load but also on the duration of treatment.

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109.2 DIFFERENTIAL AVERSIVE STIMULUS PROPERTIES OF β-PHENYLETHYLAMINE AND d-AMPHETAMINE IN RATS. A.J. Greenshaw* and C.T. Dourish* (Spon: ENA). Psychiatric Res. Div., University Hospital, Saskatoon, Sask., S7N 0X0, Canada.

Previous studies have demonstrated a number of similarities between the discriminative and reinforcing stimulus properties of β-phenylethylamine (PEA) and d-amphetamine (AMPH). Rats trained to discriminate PEA or AMPH from saline exhibit generalization between the PEA and AMPH states (Huang and Ho, 1974, Psychopharm. 35, 77-81; Goudie and Buckland, 1982, Neurosci. Lett., in press). Similarly, both compounds decrease self-stimulation thresholds and are self-administered by laboratory animals. In order to extend further the analysis of shared stimulus properties of these compounds, their effectiveness in a conditioned taste aversion (CTA) procedure was investigated. In the CTA procedure ingestion of a novel flavour followed by the administration of a drug may result in subsequent avoidance of the flavour, indicating that the drug has aversive stimulus properties. In a CTA experiment with male Wistar rats (two-bottle test, single pairing), the effects of PEA (12.5-100 mg/kg i.p.) and of AMPH (2.5 mg/kg i.p.) were compared against that of the saline vehicle. The AMPH-treated group exhibited a marked aversion to saccharin on each of four retention trials. PEA decreased saccharin intake in the 100 mg/kg group only on retention day one. Lower doses of PEA were ineffective. Doses of up to 50 mg/kg of PEA were also ineffective with a single bottle CTA procedure involving multiple conditioning trials. Higher doses were not used in the multiple pairing experiment due to fatal toxicity. These data demonstrate that behaviourally active doses of PEA are almost totally ineffective in inducing a CTA to saccharin. The stimulus properties of PEA seem to bear a closer resemblance to cocaine than to AMPH in this respect, since, despite bearing other similarities to AMPH, cocaine is a very weak CTA-inducing agent. These data could be interpreted as support for the recent proposal that endogenous PEA may mediate the stimulus properties of cocaine (Colpaert et al., 1980, Pharmac. Biochem. and Behav. 13, 513-517).

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109.3 APOMORPHINE-INDUCED PECKING IN YOUNG CHICKS: DIURNAL CYCLES. G. Marzullo and A.J. Friedhoff, Millhauser Lab., New York Univ. Sch. of Med., New York, N.Y. 10016.

The chick is a useful pharmacological model for studies relating dopamine-receptor mediated behaviors to the biochemical status of receptors binding dopamine and neuroleptic drugs. Here we describe some behavioral findings. Apomorphine (Apo) was injected (0.02-1 mg/kg, s.c.) into chicks age 3-5 days post-hatch and behavioral responses were assessed by direct observation of the birds housed singly in 21x21 cm cages made of opaque plexiglass. For assessment of running, lines were drawn subdividing the floor area into four equal squares and, for elicitation and assessment of pecking, several dots were drawn on the cage wall at chick eye level (the dots serving as conspicuous spots for the birds to peck). Peeps and other vocalizations were also scored. Chicks were injected in groups of six at 30 sec. intervals and observed for 30 sec. every three minutes for the duration of drug effect, with a cut-off at one hour.

Control chicks injected with saline frequently peeped and stepped around in the cage, and occasionally pecked at food particles or debris on the cage floor. These birds almost never pecked at the cage walls. Apo-injected chicks immediately ceased peeping and began running furiously around the cage. Within 3-5 min. the running response declined and was replaced by a rapid and vigorous pecking at the dots or other conspicuous spot on the cage walls. The pecking reached frequencies of over 100 pecks/min. and, depending on the dose of Apo, lasted up to several hours. If food was placed on the cage floor, Apo-injected chicks neglected the food and pecked only at the plexiglass wall. This response to Apo appeared mediated by dopamine receptors since a prior injection of spiroperidol or haldol (0.1 mg/kg) completely blocked pecking, as well as running responses. No other drug tested (including beta-adrenergic agonists and antagonists, serotonergic agents, opiates, scopolamine) induced pecking or appreciably modified this response to Apo.

We also found a pronounced diurnal cycle in the chick's responsiveness to Apo. This cycle became more sharply defined when embryos were incubated and chicks were reared under a 12-hour light/dark cycle (8:00 am-8:00 pm). The pecking response to a fixed dose of Apo (0.6 mg/kg) was lowest (300 total pecks) during 2 to 4 pm and increased thereafter to a maximum (2,200 pecks) occurring about 2 to 4 am. This difference did not simply reflect the birds' initial state of activity, since chicks are normally active during day time and totally inactive at night. Biochemical studies now in progress will test whether there are diurnal cycles in the density of dopamine receptor sites accompanying the behavioral changes in Apo sensitivity.

109.4 ANTAGONISM OF AMPHETAMINE-INDUCED LOCOMOTION BY ASCORBIC ACID: A PHARMACOKINETIC OR PHARMACODYNAMIC MECHANISM? M. H. Lewis, A. A. Baumeister* and R. B. Mailman. Biological Sciences Research Center, Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514

Ascorbic acid (AA) is found in high concentrations in numerous brain regions including dopamine-rich areas (Milby et al., Neurosci. Lett., 28:15, 1982). AA has been reported to antagonize amphetamine- and apomorphine-induced behavior in mice (Tolbert et al., Life Sci., 25:2189, 1979). In vitro, AA decreased dopamine-stimulated adenylate cyclase activity (Thomas & Zemp, J. Neurochem., 28:663, 1977) and the binding of DA agonists and antagonists to rat striatal membranes (Heikkila, Res. Comm. Chem. Path. Pharm., 34:409, 1981). In the present experiments, AA (1 g/kg i.p.) antagonized spontaneous as well as amphetamine-induced locomotion in three different strains of mice. Conversely, AA failed to antagonize either spontaneous or amphetamine-induced locomotion in rats. In order to determine whether such an effect was due to species differences in AA uptake into brain, AA was administered intracerebroventricularly to rats (270-540 µg). Even at such high doses AA failed to antagonize amphetamine-induced locomotion.

Species differences in concentrations of AA entering the CNS were also studied using HPLC with EC detection. Whole brain AA concentrations were determined at several time points following i.p. administration of AA (1 g/kg). These data showed small increases in AA concentrations in each species (5-10%) with slightly higher values being noted for mice. Time course data showed a maximal increase at 15 minutes for rats and at 30 minutes for mice.

The hypothesis that AA might alter the availability of amphetamine in the central nervous system was then tested. Amphetamine (20-50 µg/10 µl) was administered intracisternally 1 hour after i.p. treatment with AA (1 g/kg). Amphetamine caused similar stimulation of activity in both AA-treated and control mice. To investigate further these phenomena, pharmacokinetic studies on the effects of AA administration on the accumulation and distribution of amphetamine in rat and mouse brain are in progress.

In conclusion, the data presented above support the hypothesis that other factors, such as alterations in the pharmacokinetics of amphetamine, may be involved in the purported antidopaminergic action of AA. (Supported, in part, by PHS grants HD-03110, HD-07201 and ES-01104.)

109.5 MOOD AND SENSORIMOTOR PERFORMANCE AFTER NEUROTRANSMITTER PRECURSOR ADMINISTRATION. Harris R. Lieberman,* Suzanne Corkin, Bonnie J. Spring,* John H. Growdon*, and Richard J.Wurtman. (SPON:N.Hebben). Dept. of Psychology, MIT, Cambridge, MA 02139.

It is well established that consumption of certain foods or food constituents can change the rates at which neurons synthesize and release their neurotransmitters. Two large neutral amino acids that influence the availability of their neurotransmitter products are tryptophan and tyrosine, the precursors of serotonin and the catecholamines (dopamine and norepinephrine). Although tryptophan and tyrosine are present in most foods, little is known about their effect on normal human brain function. We therefore administered oral tryptophan (50 mg/kg) and oral tyrosine (100 mg/kg) to 20 male subjects, aged 18 to 45, using a double-blind, placebo-controlled, crossover design. The acute effects on mood and sensorimotor performance were measured. Mood was assessed by two self-report questionnaries, the Profile of Mood States (POMS), and the Visual Analog Mood Scales (VAMS), and sensorimotor performance by simple auditory reaction time (RT), two-choice visual RT, a test of fine manipulative dexterity, and a test of sensorimotor coordination.

The results for the POMS revealed that tryptophan significantly decreased Vigor ($p < .01$) and increased Fatigue ($p < .05$) when compared to either placebo or tyrosine. The other POMS scales Anger, Confusion, Tension, and Depression, were not altered by ingestion of tryptophan. In agreement with the POMS results, tryptophan significantly reduced Alertness as measured by the VAMS ($p < .01$). The other VAMS scales, Sad and Calm, were not affected. Tests of sensorimotor performance showed no change with tryptophan, except that it significantly increased simple auditory RT compared to tyrosine ($p < .05$). Simple RT measures did not distinguish the effect of either amino acid from its placebo. Tyrosine had no effect on mood or sensorimotor performance compaired to placebo.

The induction of drowsiness by tryptophan is consistent with previous reports that it reduces latency to sleep in humans and that serotonergic neurons participate in the induction and regulation of sleep. The fatigue and drowsiness associated with tryptophan administration is further evidence that this substance may be useful as a mild hypnotic. Since tryptophan unlike other hypnotics, does not seem to impair sensorimotor performance, it may have clinical utility.

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109.6 ACQUISITION OF AN ADENOSINE-SALINE DISCRIMINATION IN THE RAT: GENERALIZATION TO 2-CHLORO-ADENOSINE AND BLOCKADE BY CAFFEINE. D.G. Spencer, Jr. and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

Recent neurochemical and behavioral data on the effects of activation and blockade of adenosine A_1 receptors have suggested a direct role of adenosine in neurotransmission (Daly, J.W., et.al,Life Sci., 28: 2083, 1981; Dunwiddie, T.V. and Worth, T., J. Pharm. Exp. Ther., 220: 70, 1982). Carney and coworkers (Carney, J.M. and Coffin-Sirochman, V.L., The Pharmacologist, 23: 151, 1981; Sirochman, V. and Carney, J.M., Fed. Proc., 40: 294, 1981) have demonstrated that adenosine analogs suppress operant rates of responding and that methylxanthines with A_1 receptor affinity such as caffeine, theophylline, paraxanthine, and theobromine block this effect. It is not yet clear, however, whether this antagonism is due to interactions at the adenosine receptor or to a more nonspecific behavioral sedation-stimulation interaction. In an attempt to examine this issue, we trained food-deprived, male hooded rats to press one lever on an FR 10 schedule for food when injected with L-phenylisopropyl adenosine (L-PIA-.08 mg/kg) and to press another lever when injected with saline. Subjects required an average of 78 sessions to acquire this discrimination. Stimulus control by L-PIA was not related to suppression of lever-pressing rate but was dose-dependent, with the ED-50 being 0.03 mg/kg. Pre-session injection of 2-chloro-adenosine (2CA) was generalized to L-PIA but its potency was approximately four-fold less. Injection of caffeine at a dose of at least 5 mg/kg prior to L-PIA blocked selection of the drug lever. These results indicate that 1) a specific discrimination can be formed to adenosine, 2) A_1 adenosine receptor stimulation rather than behavioral sedation subserved the interoceptive discriminable stimuli produced by L-PIA, and 3) discriminative stimuli produced by adenosine analogues may be a useful tool for delineation of the role of adenosine receptors in brain functions.

109.7 ANTAGONISM OF CHRONIC NICOTINE ADMINISTRATION: EFFECTS ON SCHEDULE-CONTROLLED BEHAVIOR IN RATS, Victor J. DeNoble, Francis J. Ryan*, Yvonne P. Dragan*, Paul C. Mele*, John Naworal* and Richard Kornfeld*. Philip Morris Research Center, P. O. Box 26583, Richmond, Virginia 23261.

In research reported here we investigated the effects of antagonism of chronic nicotine administration on lever pressing by rats maintained under a multiple fixed-ratio fixed-interval (MULT FR FI) schedule of food presentation. Twenty-four male hooded rats were trained under a multiple schedule until responding was stable. The rats were anesthetized and an osmotic minipump filled with (-)-nicotine was inserted subcutaneously between the scapulae. Nicotine was infused subcutaneously for 240 hours (0.5 µl/hr) delivering daily doses of 8 mg/kg, 12 mg/kg, and 16 mg/kg. After 240 hours of continuous (-)-nicotine infusion the rats were challenged with the nicotinic-cholinergic antagonist mecamylamine. Blood samples (450-1000 µl) were collected from the dorsal digital vein in the hind paw under ether anesthesia the day before and the day after the mecamylamine challenge.

Characteristic performance was maintained under the MULT FR FI schedule. FI rates were significantly decreased on the first day of nicotine exposure but returned to control levels by day 2 and remained stable throughout the remainder of nicotine phase. FR rates also were decreased on day 1 of nicotine exposure but this effect failed to achieve statistical significance. Beginning on day 3, FR rates were significantly elevated on 6 out of 8 of the remaining nicotine days. Chronic nicotine treatment increased FR rates from 1.16 ± 0.12 during control sessions to 1.42 ± 0.13 responses per second during nicotine sessions. The mecamylamine challenge significantly decreased FR response rate, relative to the last three days of nicotine exposure, to 0.82 ± 0.09 responses per second. However, this decrease was not significantly different from control values. Subsequent to the mecamylamine challenge FR response rates were again significantly elevated for the remainder of the experiment. FI performance was not altered during the mecamylamine challenge.

Multiple ion detection analysis showed that levels of nicotine present in blood both before and after the mecamylamine challenge were similar, and that the blood levels (ng/ml blood) varied directly with the daily nicotine dose (8 mg/kg/day: $\bar{x}$ = 2.28 ± 0.07 SE, 12 mg/kg/day: $\bar{x}$ = 4.08 ± 0.81 SE, and 16 mg/kg/day: $\bar{x}$ = 6.21 ± 0.63 SE).

These results show that blocking nicotine's central nervous system actions following chronic nicotine treatment does not result in a disruption of scheduled-controlled performance.

109.8 SUPPRESSION OF INTERSPECIFIC AGGRESSION IN THE RAT BY LATERAL HYPOTHALAMIC INJECTION OF THE ACETYLCHOLINE SYNTHESIS INHIBITOR HEMICHOLINIUM-3. B.C. Yoburn* and M. Glusman. New York State Psychiatric Institute, New York, NY 10032.

The involvement of hypothalamic cholinergic systems in the control of mouse killing in rats is indicated by several lines of evidence. Direct injections of the cholinergic agonist carbachol into the lateral hypothalamus facilitate muricide (Bandler, Brain Res., 20:409, 1970; Yoburn et al., Pharmac. Biochem. Behav., 15: 747, 1981), while similar injections of the cholinergic antagonists atropine (Albert, Pharmac. Biochem. Behav., 12:681, 1980) and d-tubocurarine (dtc) (Yoburn & Glusman, Soc. Neurosci. Abstr. 7:271, 1981) suppress muricide. These effects are not specific to muricide as carbachol concurrently increases irritable aggression, while atropine and dtc suppress feeding. Since muricide is facilitated by stimulation of lateral hypothalamic cholinergic receptors and inhibited by blockade of these receptors, it might be expected that inhibition of acetylcholine (ACh) biosynthesis would suppress muricide. The present experiment examined this possibility using intrahypothalamic injections of hemicholinium-3 (HC3) which depletes brain ACh following intraventricular administration (Freeman et al., J. Pharmac. exp. Ther., 210:91, 1979).

Six, male, food-deprived rats that spontaneously killed mice were implanted with a 22ga guide cannula aimed at the right lateral hypothalamus. Following recovery, 0.5µl injections of 0.9% saline or HC3 (20,30µg) dissolved in saline were administered through a 28ga injection cannula. Injection order was counterbalanced and at least 6 days elapsed between injections. The latencies to attack and kill a mouse and the rats' response to handling (irritability) were measured 1,2,3,24,48 and 72 hr postinjection. HC3 produced a dose-dependent increase in the latencies to attack and kill ($p < .05$) during the first 3 hr postinjection, but no difference ($p > .05$) 24-72 hr postinjection. A decrease in irritability that had a time course similar to the effects on muricide was also observed.

The presumed depletion of lateral hypothalamic ACh by HC3 resulted in suppression of muricide and a concurrent decrease in irritability. These data are complementary to previous results that show that intrahypothalamic carbachol facilitates muricide and irritability, and cholinergic antagonists suppress muricide. Thus, muricide and irritability, as well as other motivated behaviors, appear to be dependent upon activity in lateral hypothalamic cholinergic systems.

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109.9 YOHIMBINE HAS PARTIAL ANTIDOPAMINERGIC AND LYSERGIC ACID DIETHYLAMIDE-ANTAGONISTIC ACTIVITY ONLY AT NONSELECTIVE, BEHAVIORALLY ACTIVE DOSES. B.S. Neal*, L.P. Dwoskin* and S.B. Sparber* (SPON: R. Messing). Dept. of Pharmacol., Univ. of Minnesota, Minneapolis MN 55455.

Low doses of yohimbine (0.25-1.0 mg/kg, ip), which do not alter fixed ratio (FR) behavior in rats, are capable of antagonizing the suppression of FR behavior caused by the α_2-receptor agonist, clonidine (JPET, in press). However, since clonidine (1.0-300 µg/kg, ip) could not antagonize the behavioral suppressant action of yohimbine (2.5-5.0 mg/kg, ip), it is reasonable to believe that the behavioral action of yohimbine is due to effects at other receptor sites. It has been suggested that yohimbine blocks dopamine (DA) receptors (JPET 215, 494, 1980) and stimulates serotonin (5-HT) receptors centrally (Eur. J. Pharmacol. 15, 318, 1971). The effects of apomorphine on FR behavior can best be described as curvilinear, supporting the notion that low doses of apomorphine act on a different population (i.e. mainly presynaptic) than do high doses (i.e. postsynaptic). The former action probably accounts for apomorphine's efficacy in Huntington Chorea patients (Life Sci. 15, 1371, 1974). The behavioral effects of lysergic acid diethylamide (LSD) are generally attributed to an action on at least one type of 5-HT receptor, which makes it a good candidate for testing the hypothesis that yohimbine also acts centrally at 5-HT receptors. Drug interaction experiments revealed that behaviorally inactive doses of yohimbine (0.25-0.5 mg/kg) were incapable of altering the effects of 0.1 or 0.5 mg/kg apomorphine or 0.05 and 0.1 mg/kg LSD. Behaviorally active doses of yohimbine (2.5-5.0 mg/kg) significantly antagonized the effect of the high dose of apomorphine only and antagonized the effect of LSD (50 µg/kg) on FR behavior. The antagonism was only partial and occurred at similar doses of yohimbine. It is concluded that behaviorally active doses of yohimbine (>1.0 mg/kg) are devoid of selective pharmacologic action as α_2-antagonists and should not be used as such for pharmacological studies in which only α_2-antagonistic properties are sought. Supported in part by U.S.P.H.S. grants DA 00532 and GM 07397.

109.10 A NEW LOOK AT THE STIMULUS PROPERTIES OF THE SEDATIVE-HYPNOTIC CLASS OF DRUGS USING A THREE-CHOICE DRUG DISCRIMINATION PARADIGM. J.L. Howard and S.T. McBennett*. Department of Pharmacology, Burroughs Wellcome Co., Res. Tri. Park, N.C. 27709.

A recent review of the stimulus properties of the sedative-hypnotic-anxiolytic class of drugs concluded that the relationships among the drugs in this classification was unclear (Barry and Krimmer, Neuropharmacology, 1979, 18, 991). Based on results from two-choice drug discrimination experiments using either drug-no drug or drug-drug training procedures, no definitive statement is possible regarding the number of stimulus dimensions or the generalization patterns in this broad class; training conditions seem to determine outcome.

In our work, 22-hr. food-deprived, male Long-Evans rats were trained in Coulbourn operant chambers to select one of three levers to press for food reward on a FR-10 schedule depending upon which of three kinds of injections was administered 15 min. prior to the session. The training conditions were chlordiazepoxide (CDP), 5 mg/kg i.p., vs pentobarbital (PENT), 10 mg/kg i.p., vs saline; CDP 5 mg/kg i.p. vs PENT, 20 mg/kg i.p., vs saline; CDP 5 mg/kg i.p. vs PENT, 20 mg/kg i.p., vs saline; CDP, 5 mg/kg i.p., vs ethanol (ETOH), 1g/kg i.p., vs saline; CDP, 7.5 mg/kg p.o., vs PENT, 15 mg/kg p.o., vs saline; and PENT, 15 mg/kg p.o., vs ETOH, 1.5 g/kg p.o., vs saline. All training conditions were learned to a criterion of completion of the first FR-10 on the appropriate lever in less than 12 total responses for 9 of 10 consecutive sessions in approximately 90-110 sessions. Following completion of training, dose-response curves for CDP, PENT and ETOH were determined in each group prior to gathering data from other drugs.

The results indicate that a qualitative difference exists among the stimulus states induced by the three prototype drugs used. For example in the rats trained to discriminate CDP/PENT/Sal, at low doses of either drug, responding was on the saline lever switching to the drug-appropriate lever as dose increased up to and beyond the training dose. Most responding was on the saline lever following ETOH treatments. Barbiturates and meprobamate induced responding on the PENT lever; benzodiazepines on the CDP lever. Morphine, chlorpromazine, dilantin, and adenosine induced saline-lever responding.

Thus the results to date indicate that the three-lever drug discrimination paradigm yields useful information in excess of that provided by the two-choice task.

109.11 INDEPENDENCE OF THE ANTIAVERSIVE ACTION OF GABAERGIC AGENTS FROM BENZODIAZEPINE RECEPTORS. K.G. Lloyd, Ph. Bovier* and C.L. Broekkamp*, LERS-SYNTHELABO, 31 ave P.V. Couturier, F. 92220 Bagneux, France.

It is widely accepted that benzodiazepines (BZDs) exert their anticonvulsant and anxiolytic actions via a specific BZD receptor which is an integral part of a BZD-GABA macromolecular complex (Costa, Arzn. Forsch, 30, 858, 1980). If this is correct, then GABA mimetics should also be active in anxiolytic situations, like the BZDs. In this regard electrical stimulation of the periaqueductal grey region (PAG) in the rat induces an escape reaction which is quantifiable in terms of latency (secs) and threshold (µA) (Bovier et al, Eur. J. Pharm. 75, 77, 1981). Diazepam increases the latency and the threshold in a dose-dependent manner (7 rats, vehicle = 6 ± 9 sec.; 5 mg/kg, ip = + 60 ± 13 secs ; 7.5 mg/kg, ip = + 102 ± 8 secs). Progabide, a GABA agonist, and sodium valproate (a GABA mimetic) had qualitatively and quantitatively similar effects. Thus, at 100 mg/kg, ip progabide increased latency (from + 6 ± 7 to 71 ± 8 secs) and threshold (from + 5 ± 3 to 32 ± 4 µA) and 400 mg/kg, ip of sodium valproate was equally effective (from + 8 ± 4 secs to + 99 ± 12 secs). At these doses, the spontaneous locomotor activity was unaltered. In contrast, diphenylhydantoin, at doses up to 50 mg/kg, ip, was inactive, and haloperidol was effective only at doses which markedly interfered with locomotor activity. Even at a cataleptic dose of haloperidol (0.5 mg/kg) which greatly increased the escape latency, the PAG stimulation still evoked piloerection and vocalization. In contrast, progabide and diazepam blocked these in parallel with the increase in escape latency. The coadministration of inactive doses of diazepam (2.5 mg/kg, ip) and progabide (25 or 50 mg/kg, ip) resulted in highly significant increases in escape latency and threshold (diazepam, 2.5 mg/kg = 12 ± 8 secs ; progabide, 50 mg/kg, = + 12 ± 9 secs ; diazepam and progabide = + 74 ± 8 secs). The effects of both diazepam and progabide were related to GABA receptor activity as bicuculline (3 mg/kg, ip, inactive per se) blocked equally the effect of both the BZD (reduction from + 87 ± 17 to + 26 ± 7 secs) and the GABA agonist (from + 90 ± 11 secs to + 52 ± 5 secs). In contrast, RO 15-1788, a specific antagonist of BZD receptors, (Hunkeler et al, Nature, 290, 514, 1981) was inactive vs progabide while completely reversing the action of diazepam. These results support the GABA receptor complex hypothesis for the action of BZDs and suggest that anxiolytic agents may be developed independently of an action on BZD receptors.

109.12 ANTAGONIST PRECIPITATED WITHDRAWAL FOLLOWING CHRONIC CHLORDIAZEPOXIDE DOSING IN THE RAT. N. Boisse, M.Gay*, J. Guarino*, H. Kruger* and G.Ryan*. Section of Pharmacology, Northeastern University, Boston, MA 02115.

RO 15-1788 (R/A), a selective antagonist of the CNS effects of classical anxiolytic-sedative-hypnotic benzodiazepines (BNZ), is reportedly devoid of intrinsic activity and antagonizes BNZ actions by displacing BNZ from its receptor. To test the hypothesis that BNZ receptors are important in the expression of BNZ physical dependence, we have evaluated the effects of R/A following the induction of tolerance and dependence to chlordiazepoxide (CDP) by "chronically equivalent" maximally tolerable dosing for 5 weeks. CDP was administered i.g., b.i.d., in doses individually adjusted to produce an equivalent motor impairment (severe ataxia). The mean final dose was 435 mg/kg. Stopping treatment gives a spontaneous withdrawal (SWD) characterized by 16 motor, autonomic and behavioral signs. WD signs were rated (0-3) by 4 independent raters. WD score is the mean summed rating. Onset of SWD was 2-5 days; SWD peaked on day 8 (WD Score = 26.4 ± 0.8 SE) and disappeared by 14 days. R/A (5-100 mg/kg, i.p.) on day 8 of SWD had no effect. R/A gave the most intense precipitated withdrawal (PWD) 24-30 hrs post chronic treatment. The threshold dose of R/A was 10 mg/kg; maximum intensity was at 25 mg/kg (standard dose). R/A caused a PWD that was qualitatively similar to SWD with 4 more signs: salivation, spread eagle posture, spasticity and rigidity of claws, and diarrhea. Onset was 1 min, peak intensity (WD score = 19.5 ± 5.1) was at 5 min; WD disappeared within 5 hrs. Twelve to 24 hrs later rats were more intoxicated than prior to R/A suggesting partial loss of BNZ tolerance. In one rat, R/A precipitated a forelimb clonic seizure lasting 2 min. PWD (19.5 ± 5.1) was less severe than SWD (26.4 ± .8) suggesting that specific BNZ receptor mediated events account for only a part of the SWD. To more selectively induce dependence at the BNZ receptor, lower fixed doses of CDP (20, 40 & 75 mg/kg) were administered b.i.d. for 5 weeks. When R/A was given 4-6 hrs. post-treatment, a PWD qualitatively similar to high dose PWD was seen. PWD scores were similar for 40 & 75 mg/kg treatments (19.2 ± 0.8; 19.6 ± 1.1) and less for 20 mg/kg treatment (16.5 ± 2.0) suggesting a ceiling effect. The SWD score for 75 mg/kg treatment (19.1 ± 1.3) was similar to PWD scores for 75 mg/kg and maximally tolerable high dose but less than for high dose SWD. These findings support the hypothesis that the SWD following chronic low dose BNZ involves specific receptors but the SWD following chronic high dose treatment involves both specific receptors and some other mechanism(s). (Supported in part by NIDA Grant DA-02398 and by BRSG Grant SO7-RR 05830-02).

109.13 POIKILOTHERMIA OF ETHYL ALCOHOL AND THE POSSIBLE ROLE OF BRAIN SEROTONIN. Shahid Salles, M.S., K. Sharifi Hossaini and M. Varedi
Department of Physiology, School of Medicine, Shiraz University, Shiraz, Iran.

Ethyl alcohol, 3.5 g/Kg or physiological saline, was administered intra gastrically to different groups of male and female rats (N = 10 per group). Animals were exposed to room (22°c), cold (8°c) and warm (32°c) temperatures before or after alcohol administration. All rats stayed at least 2 hours at each environmental temperature before being transferred to the next chamber. A therimster inserted for at least 5-6 cm into the animals' colon was used to record the body temperature during the experiment. In some rats p-chlorphenylalanine (PCPA) 100 mg/KgmIP was given for 48 hrs. before the experiment in order to reduce the 5-HT content of the CNS. Ethyl alcohol reduced the body temperature at room temperature, and at 8°c and more so in animals pretreated with PCPA. Heat exposure (32°c) raised the body temperature in the following order; alcohol treated > alcohol in PCPA treated rats > saline treated. The results indicate the poikilothermic effect of ethyl alcohol in the dose administered in rats and the possible interaction of ethyl alcohol and the brain 5-HT during heat and cold exposures.

109.14 EFFECT OF VITAMIN C ADMINISTRATION ON THE EEG AND BEHAVIORAL ACTIONS OF MORPHINE IN THE GUINEA PIG. Sharifi Hossaini, K. and M.S. Shahid Salles. Departments of Pharmacology and Physiology, School of Medicine, Shiraz University, Shiraz, Iran.

Guinea pigs (400-500 gm) were prepared with permanent electrodes for recording EEG and EMG. Injection of morphine HCl 10 mg/Kg subcutaenously was followed by the appearance of high voltage EEG slow bursts which were associated with stuporous behavior. This phase was suppressed by the appearance of behavioral arousal evidenced by EMG recordings and changes such as squeaking. Afterwards behavioral sleep became apparent on the EEG. Administration of morphine to guinea pigs pretreated with vitamin C (20 mg/guinea pig/day, I.P.) for 3 days, the last one 4 hrs before the morphine injection, was followed by a significant increase in duration of high voltage EEG slow bursts and prolonged sleep onset. However, duration of hyperarousal did not change significantly in comparison with the controls. These findings indicate the enhancing effect of vitamin C on morphine actions at the dose used in these experiments. This action may be mediated through binding with opiate receptors in the brain that can be potentially beneficial for the treatment of narcotic addiction.

1- Dunlap, C.E., et al. Molec. Pharmacol. 16:105-119, 1979.

2- Libby, A.F. and I. Stone, Orthomolec. Psychiatry 6:300-308, 1977.

110.1 COMPARISON IN SPINAL NEURONS OF MORPHOLOGIC AND CHOLINERGIC FACTOR ACTIVITIES FROM MUSCLE EXTRACT in vitro, R.G. Smith,* and S.H. Appel, (SPON: H.F. Epstein), Dept. of Neurology, Baylor College of Med., Houston, TX 77030.

Motor neurons in vitro require the presence of either muscle in co-culture, medium conditioned on myotubes, or extracts of skeletal muscle for continued survival and differentiation. A high-speed aqueous supernatant extracted from rat forelimb muscle (MX) has been previously reported to induce changes in the morphology of dissociated ventral spinal neurons, and in the cholinergic activity of motor neurons in culture. Using neurite outgrowth as a morphologic index of neuronal differentiation, we have compared the effects of MX on these two parameters of development.

The addition of MX to cultures of dissociated 14-day embryonic rat ventral spinal cord stimulates both morphologic and cholinergic differentiation in similar concentration and time dependent fashions. The ability of MX to increase the amount of acetylcholine synthesis and process outgrowth is stable to freezing and lyophilization, and labile to heating (60°, 1 hr) and protease digestion with trypsin and papain. Both activities are recovered in the 35-60% $(NH_4)_2SO_4$ fraction, and are present in the 0.18-0.35 M NaCl gradient fraction from a DEAE column.

MX induced neurite outgrowth only in neuronal systems that normally contain cholinergic cells (fetal ventral spinal cord, ciliary ganglion, and paraspinal sympathetic ganglia, but not dorsal root ganglia or dorsal spinal cord). The morphologic activity of the extract appears to be specific to skeletal muscle since extracts from other tissues do not promote neurite extension. The cholinergic activity, however, is also stimulated by extracts from other cholinergic tissues (e.g. heart and cerebral cortex). Although simple characterization and partial purification fail to separate the factor activities, they appear to be regulated differently. The ability of MX to promote neurite extension in vitro decreases significantly as a function of the age of the animal from which the muscle supernatant is isolated. Cholinergic regulation by MX is not under such a pronounced age dependent control. This result, in conjunction with the different tissue specificities for cholinergic and morphologic activities, suggests that more than one factor may be involved. (Supported by grants from the John A. Hartford Foundation and the Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation)

110.2 STIMULATION OF DIFFERENT NEURONAL POPULATIONS IN CILIARY GANGLIA BY STRIATED MUSCLE-CONDITIONED MEDIUM AND CYCLIC AMP. G.M. Monastersky* and F.J. Roisen. Department of Anatomy, UMD-Rutgers Medical School, Piscataway, NJ 08854.

The chick ciliary ganglion (CG) is a parasympathetic ganglion containing two distinct types of cholinergic neurons: 1) large ciliary neurons which innervate striated muscle, and 2) smaller choroid neurons which innervate smooth muscle. Explanted CG extend neurites only in medium that has been conditioned by striated muscle or contains striated muscle extracts. We have observed that cyclic adenosine 3',5'-monophosphate (cAMP) (0.5mM to 30mM) and its dibutyryl derivative elicit increases in number and length of neuronal processes radiating from organized cultures of 11 day embryonic chick CG. The neuritic growth patterns produced by these nucleotides and those obtained by treatments with striated muscle-conditioned medium (SCM) were different. Neurite development produced by SCM-supplemented media was dependent on contact with a non-neuronal substratum, while cAMP-stimulated neurite extension was not. To determine which of the two neuronal cell types was stimulated by each of these treatments, trypsin-dissociated cultures of 11 day embryonic chick CG were fed medium (Basal Medium Eagle with 10% heat-inactivated fetal calf serum and 2.2 g/l $NaHCO_3$) supplemented with one or more of the following test agents: SCM, 20mM cAMP, 20mM theophylline, 10mM dibutyryl cAMP, and 10mM Na butyrate. All cultures were grown on collagen-coated, 35mm dishes for 30h at 35°C prior to microscopic evaluation. The mean number and length of neurites exhibited by the two neuronal populations were determined for each treatment. SCM appeared to stimulate only the large ciliary neurons; no morphological effect on the smaller choroid cells was observed. In contrast, treatment with cAMP-stimulated neurite formation from the smaller choroid cells with only occasional stimulation of the ciliary neurons. Simultaneous treatment with SCM and cAMP produced neurites from both cell types. Addition of theophylline to this double-treatment group increased the number of choroid cells extending neurites; theophylline alone selectively stimulated choroid cell neurite development. Although Na butyrate and dibutyryl cAMP stimulated neurite production from both cell types, the nucleotide had a greater effect on the choroid cells. These results suggest that SCM and cAMP act on different types of neurons in the CG and that SCM appears to stimulate only those neurons which normally innervate striated muscle. Furthermore, it is likely that the SCM-stimulation of ciliary neurons is not mediated by cAMP. Supported by NIH grant NS 11299.

110.3 ANTISERUM AGAINST A FACTOR THAT STIMULATES CHOLINERGIC DEVELOPMENT OF CHICK CILIARY GANGLION NEURONS. Jes Stollberg and Darwin K. Berg. Department of Biology, Univ. of Calif., San Diego; La Jolla, CA. 92093

The chick ciliary ganglion contains cholinergic neurons that innervate smooth and striated muscle in the eye. Previous work has shown that extracts prepared from eye tissue contain two activities that promote the long-term development of the neurons in dissociated cell culture. The activities can be separated by gel filtration: one selectively stimulates neuronal growth (Growth-Promoting Activity, GPA) while the other one selectively stimulates cholinergic development (Choline acetyltransferase Stimulating Activity, CSA). We have obtained a rabbit antiserum against CSA that specifically blocks the effect of CSA on the neurons in cell culture.

The antiserum was produced by injecting a rabbit with crude fractions of CSA obtained by gel filtration of eye tissue extracts. The immune serum was tested by titrating it against CSA in culture medium and supplying cultures with the mixture for 7-9 days. In this paradigm CSA alone induces a 2-4 fold increase in choline acetyltransferase levels over those found in control cultures lacking tissue extracts. The immune serum at a dilution of 1:1000 completely blocks the stimulatory effect of CSA, resulting in levels of choline acetyltransferase equivalent to control values. The antiserum does not diminish the low levels of choline acetyltransferase found in cultures grown without tissue extracts, and it has no effect on neuronal growth with or without CSA. Pre-immune serum at a dilution of 1:25 does not block the effect of CSA. Neuronal survival is complete and neuronal growth rates are equivalent in all cases. IgG purified from the immune serum by ammonium sulfate precipitation and ion exchange chromatography has the same effect and specificity as the whole antiserum.

CSA has been purified 300 fold over the levels found in embryonic chick eye extract by starting with eye tissues of high specific activity and fractionating the extracts by gel filtration and ion exchange chromatography. The recovered material gives half-maximal stimulation of neuronal cultures at 50 ngm protein per ml medium. The partially purified CSA is heat labile: 3 min at 100° destroys the activity. SDS gel electrophoresis reveals many protein components in the material, indicating that it is still far from pure.

The antiserum should be useful in a further characterization of CSA and may permit an examination of its effects in vivo. (Supported by NIH grant NS 12601).

110.4 NEURITE GROWTH PROMOTING FACTOR(S) PRODUCED BY THE CNS OF HELISOMA. R. G. Wong, D. L. Barker, S. B. Kater, D. A. Bodner* and G. C. Hauser.* Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

Previous work in our laboratory has shown that brain-conditioned medium (CM) is essential for outgrowth of isolated neurons of the snail Helisoma (Wong et al., J. Neurosci. 1:1008, 1981). This brain-derived conditioning factor(s) (CF) was reported to be a noncellular, soluble substance(s) that binds to tissue culture substrates and whose action is not mimicked by NGF, fibronectin or sera.

We now report that the production of CF appears to be specific to neural tissue. Only central and buccal ganglia, and not blood, buccal mass, esophagus, salivary gland or penis, were capable of producing or releasing a CF which stimulated neurite outgrowth. The time course for the appearance of CF activity suggested that ganglia conditioning the medium required 24-72 hours to produce (or release) neurite growth-promoting factor. At shorter periods (e.g. 12 hours) growth-promoting activity was not detectable. The only morphological change caused by 12 hr CM was the initiation of veiling.

It is possible that there is physiological regulation of CF release and/or production. The number of cells that grew neurites was approximately linear with the amount of neural tissue used to condition the medium up to 2 ganglionic rings/ml. Addition of more ganglia failed to stimulate a greater response. This plateau of CM activity appears to be a function of production and/or release of CF, rather than a limited response, since dose-response curves for dilutions of CM were approximately linear regardless of the number of ganglia used for conditioning.

Preliminary characterization of CF indicates it contains a proteinaceous component. CF activity was destroyed by trypsin, chymotrypsin, and heating to 100°C, but not by DNAse nor RNAse. In addition, anisomycin inhibited 35% of CF production under conditions where 90% of total protein synthesis was blocked in cultured central ganglia. Under these conditions, anisomycin had no apparent effect on the maintenance of electrical excitability or choline metabolism in cultured buccal ganglia.

Since CF stimulates neurite outgrowth in ganglia as well as from isolated neurons, and since ganglia appear to synthesize, store and release CF, this factor(s) may be important for the normal regulation of CNS growth. (Supported by NS 15350.)

110.5 NEURITE OUTGROWTH AND CHOLINE METABOLISM ARE ENHANCED BY SEPARATE FACTORS PRODUCED BY THE CNS OF HELISOMA. D. L. Barker, R. G. Wong, S. B. Kater and B. J. Bullard.* Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

Neurons from the snail Helisoma require a brain-derived factor(s) for neurite growth in both organ and isolated cell culture. This growth-promoting activity is released from central ganglionic rings cultured in defined medium, producing brain-conditioned medium (CM) (Wong et al., J. Neurosci. 1:1008, 1981). A search for metabolic correlates of growth has revealed that CM 1) enhances the incorporation of ^{3}H-choline into specific metabolites, but that 2) this choline metabolism-enhancing factor(s) is distinct from neurite growth-promoting factor(s).

Buccal ganglia were cultured in CM or defined (unconditioned) medium for 3 days and then uptake and metabolism of ^{3}H-choline were determined as described previously (Hadley et al., J. Neurobiol. 13:217, 1982). CM enhanced incorporation of ^{3}H-choline into acetylcholine (1.7 fold), phosphorylcholine (4.4 fold) and lipid (3.1 fold). Total uptake of ^{3}H-choline was increased 1.7 fold but the amount of free ^{3}H-choline in the tissue was not significantly changed. Although it is not known whether CM affects simply the rate of choline uptake or a number of specific enzyme activities, it is clear that CM enhances choline metabolism as well as promoting neurite outgrowth.

Further investigation suggested that different factors are responsible for promoting growth and enhancing choline metabolism. When central ganglia were treated with 20 μM anisomycin during the production of CM, the choline metabolism-enhancing activity was completely absent from the resulting CM while the growth-promoting activity was reduced by only 35%. (Total protein synthesis was inhibited by over 90% in 20 μM anisomycin.) While this result suggests the presence of two (or more) distinct factors, a clear demonstration of different factors requires a physical separation of the two activities. We have accomplished this by taking advantage of the fact that the growth-promoting factor(s) can be removed from CM by adsorption to a polylysine-coated surface. Removal of over 95% of the neurite growth-promoting activity from CM had no effect on its abiltiy to enhance choline metabolism. These results suggest that the Helisoma nervous system produces a variety of humoral factors that are involved in regulating the complex interactions between neuronal growth and metabolism. (Supported by NS 15350.)

110.6 INDUCTION BY INJURY OF NEURONOTROPHIC ACTIVITY IN RAT BRAIN: CORRELATION WITH SURVIVAL OF IMPLANTS IN THE WOUND CAVITY. M.Nieto-Sampedro, M.Manthorpe, S.D.Skaper, E.R.Lewis, G.Barbin, F.M.Longo*, S.Varon and C.W.Cotman. Dept. of Psychobiology, Univ. California, Irvine, CA 92717 and Dept. of Biology, School of Medicine, Univ. California, San Diego, CA 92093.

A cavity was aspirated in the entorhinal/occipital cortex of developing or adult rats and a small fragment of Gelfoam^R was placed there in order to collect the fluid secreted into the wound. When this fluid was assayed for its ability to support the 24h survival of dissociated neurons in culture it was found that its content of substances with neuronotrophic activity increased over time. Trophic activity was very low or absent in non-injured brain, increased sharply over the first 3-6 days following the lesion and decayed after reaching a maximal level (about 15 days in the adult). The factor/s supported the survival in culture of dissociated neurons from chick embryo spinal cord, sympathetic ganglion, dorsal root ganglion and ciliary ganglion, as well as mouse dorsal root ganglion. Another substance with growth interfering properties for spinal cord neurons was induced concurrently with the trophic activity. The neuronotrophic factors were non-diffusible and heat and trypsin sensitive-- three properties consistent with a protein nature, and their activity was not affected by an antibody to conventional nerve growth factor.

Fragments of rat embryo corpus striatum placed in a wound cavity in the entorhinal/occipital cortex of neonate rats immediately after making it showed very poor survival and no innervation of the host hippocampus. However, if implantation was delayed 3-6 days after making the cavity, survival was greatly enhanced and innervation of the host tissue took place. The time course for the accumulation of trophic factor/s paralleled the delay leading to increasing graft survival and innervation. It seems likely that the neuronotrophic activity that accumulated in the wound cavity during the delay period was responsible for the increased survival of the implants.

110.7 INTRAVENTRICULAR INJECTIONS OF NERVE GROWTH FACTOR (NGF) INCREASE THE ACTIVITY OF CHOLINE ACETYLTRANSFERASE (CAT) IN THE BRAIN OF NEWBORN RATS. F. Hefti, H. Gnahn*, R. Heumann*, and H. Thoenen. Max-Planck-Institute for Psychiatry, Dept. Neurochemistry, Martinsried, West Germany.

NGF, injected into the rat hippocampus, is taken up by terminals of presumptive afferent cholinergic neurons and is transported retrogradly to their cell bodies located in the nucleus of the diagonal band of Broca (Schwab et al., Brain Res. 168, 473, 1979). In the peripheral nervous system, retrograde transport of NGF is characteristic for sympathetic and spinal sensory neurons, which are dependent on NGF for their normal development and maintenance of function. A possible physiological role of NGF for central cholinergic neurons is also implied by the observation that lesion of the cholinergic projection to the hippocampus, evokes the sprouting of axons of peripheral sympathetic neurons into the area previously innervated by cholinergic terminals (Crutcher and Davis, Trends Neurosci. 3, 70, 1981).

To further establish a possible role of NGF on central cholinergic neurons, we injected NGF intraventricularly into newborn rats and measured the activity of CAT in various brain areas. Starting at the second postnatal day, 30 μg of 2.5S NGF (isolated from mouse submandibular glands) were injected every second day during a period of 8 days. Controls were injected with equal amounts of cytochrome C. NGF treatment resulted in 80% increase in CAT activity in the septum, 30% in the hippocampus, and 70% in the cortex. Similar increases were obtained with NGF isolated from bovine seminal plasma. Bovine NGF is free of renin activity, and these results therefore indicate that the renin contamination of mouse NGF is not responsible for the stimulations of CAT activity in the brains of newborn rats.

These results are consistent with a trophic role of NGF on central cholinergic neurons. However, it remains to be established whether the normal development of central cholinergic systems and the ingrowth of peripheral sympathetic fibers into the hippocampus after septial lesions are impaired by neutralization of endogenous NGF.

110.8 A β NERVE GROWTH FACTOR ISOLATED FROM THE HIGH MOLECULAR WEIGHT NGF OF MASTOMYS NATALENSIS. T.L.J. Darling* and E.M. Shooter. Dept.of Neurobiology, Stanford Univ.Sch. of Med. Stanford,CA94305

The nerve growth factor (NGF) activity of the submaxillary glands of male and female Mastomys natalensis has been shown to reside primarily in a complex having a molecular weight of 75,000 by sucrose density gradient sedimentation. The complex has been purified by chromatography on Sephadex G-100, DEAE cellulose, and BioGel P-100. Analysis of the proteins in the complex by non-reducing SDS polyacrylamide gel electrophoresis reveals 3 peptides, which have molecular weights of 70,000; 27,000; and 13,000. The latter has been identified as a βNGF protein. Chromatofocusing of the NGF complex between pH7 and pH4 results in the separation of the bulk of the 70,000 peptide from the 27,000 and βNGF peptides, which co-purify. The βNGF subunit has been isolated by disruption of the complex at pH 3.6 in the presence of 3 M urea, followed by chromatography on sulphopropyl Sephadex with elution by a pH gradient. The isolated βNGF is pure on the basis of isoelectric focusing in the presence of urea on polyacrylamide gels, and SDS polyacrylamide gel electrophoresis. The Mastomys βNGF has a pK identical to that of mouse βNGF, and is identical in size when subjected to electrophoresis in SDS under reducing and non-reducing conditions. The amino acid analysis of Mastomys βNGF is similar to that of mouse βNGF. When subjected to isoelectric focusing in the presence of urea, the Mastomys βNGF exhibits a variable amount of a slightly less basic form, analogous to the des-Arg^{118}βNGF of mouse, in which the carboxy terminus arginine has been removed by the action of carboxypeptidase B or a similar enzyme in the submaxillary gland. The Mastomys βNGF has a dose response curve for neurite outgrowth from chick dorsal root ganglia neurons identical to that of mouse βNGF.

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110.9 ENDOGENOUS NERVE GROWTH FACTOR (NGF) IS RETROGRADELY TRANSPORTED IN SYMPATHETIC NEURONS IN VIVO. M. A. Palmatier*, B. K. Hartman and E. M. Johnson. Depts. of Pharmacology and Psychiatry, Washington Univ. Sch. of Med., St. Louis, MO 63110.

NGF is generally regarded as a physiologically important trophic factor for some sympathetic and sensory neurons. The mode of action appears to be its elaboration by peripheral targets of the neuron and subsequent retrograde transport to the cell body. Despite the fact that many cells secrete NGF when placed in tissue culture, peripheral tissues in vivo contain NGF levels below the limit of detection by current technology (Harper, G.P. and Thoenen, H., J. Neurochem. 34:5, 1980; although see Ebendal, T. et al., Nature 286:25, 1980). Hence, current data show that under some conditions target tissues can make NGF and that ^{125}I-NGF can be transported but direct evidence is lacking that NGF is made in vivo in normal targets and is retrogradely transported to the cell body.

To attain a more direct demonstration, the superior postganglionic nerve trunks exiting the superior cervical ganglia (SCG) of male guinea pigs (GP) were ligated. Twenty hours later the animals were perfused, and frozen sections were prepared encompassing the SCG and the nerve pre- and post-ligation. The tissues were stained with a rabbit antiserum against purified GP-NGF ("β-NGF") and several normal rabbit sera followed by FITC conjugated anti-rabbit Ig. Bright fluorescence was observed only distal to the ligation using anti-GP-NGF. Passing this antiserum over a 2.5 S mouse NGF affinity column removed the ability of the serum to stain ligated axons. The distal side of the ligated nerve stained with antibodies eluted from the 2.5 S mouse NGF affinity column. The staining properties of the various antiserum preparations correlated with their biological activity in the dorsal root ganglia bioassay, unabsorbed antiserum blocked neurite outgrowth in the NGF bioassay while the absorbed serum did not.

These experiments provide strong evidence that endogenous NGF is made in peripheral target tissues and is retrogradely transported in vivo.

110.10 NERVE GROWTH FACTOR REGULATES PEPTIDE CONTENT IN PRIMARY SENSORY NEURONS.

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Nerve growth factor (NGF) is a protein essential for the development of primary sensory neurons. Treatment of newborn rats with NGF results in a significant increase in substance P (SP) and somatostatin (SOM) levels in dorsal root ganglia and dorsal spinal cord. The physiological importance of endogenous NGF for the development of SP- and SOM-containing neurons is demonstrated by the effects of anti-NGF antibodies: Application of either purified heterologous or monoclonal anti-NGF antibodies resulted in a marked reduction of the contents of both peptides.

In addition, the deleterious effect of capsaicin on SP- and SOM-containing sensory neurons in newborns could be partially antagonized by the simultaneous administration of NGF.

These results show that NGF is a trophic agent for SP- and SOM-containing primary sensory neurons. Since these neurons represent only subpopulations of the dorsal root ganglia neurons it is conceivable that NGF regulates other peptidergic neurons as well. This assumption is supported by our immunohistochemical findings showing that the content of vasoactive intestinal polypeptide- and cholecystokinin-like immunoreactivity is increased by exogenous NGF in both cell bodies and processes.

This work was supported by the Swiss National Foundation for Scientific Research (Grant No. 3.077-0.81)

110.11 SUBSTANCE P, A MARKER OF SENSORY RESPONSIVENESS TO NERVE GROWTH FACTOR IN CULTURE. J.E. Adler, J.A. Kessler and I.B. BLACK. Division of Developmental Neurology, Cornell University Medical College, New York, N.Y. 10021.

We have previously reported that substance P (SP) rises 3-fold in neonatal rat dorsal root ganglion (DRG) explants cultured in the presence of fetal calf serum. However, SP levels were not affected by addition of NGF to the medium. To investigate potential mechanisms for the rise in SP, and to further examine the role of NGF in sensory regulation, we have now extended our observations to two additional sensory culture systems.

Neonatal rat DRG explants were grown on reconstituted collagen in a serum-free, chemically defined medium, containing transferrin, insulin, putrescine, selenium and progesterone. In all cultures, neurites initially appeared between 24 and 48 hours and continued to extend growth cones for two weeks, the length of the culture period. In defined medium, ganglia grown without added NGF failed to show a rise in SP at one week, while addition of NGF (10u/ml) increased SP content from 35 ± 3 to 70 ± 4 pg. Thus, the use of serum-free medium revealed NGF-responsiveness, which may have been obscured by NGF-like activity in serum itself or produced by serum-dependent ganglion cells.

To determine whether the NGF-dependent rise in SP was due to increased neuronal survival or to increased SP content per neuron, ganglia were dissociated and grown in defined medium at a density of 5,000 to 10,000 neurons per dish. After 24 hours of incubation, cultures without added NGF contained 50 ± pg of SP per dish. The addition of NGF (2 to 100u/ml) increased cell survival by 60% while SP rose to 80 ± 5 pg per dish. Consequently, in culture, NGF apparently increases SP by enhancing neuronal survival. However, since only a minority of DRG neuons contain SP, data based on total neuronal survival must be interpreted with caution. (Supported by NIH grants NS 06239, NS 10259 and ND 12108.)

110.12 SUBSTANCE P LEVELS DIFFER IN SYMPATHETIC TARGET ORGAN TERMINALS AND GANGLION PERIKARYA. W.O. Bell,* J.A. Kessler, and I.B. Black (SPON: K. Markey). Division of Developmental Neurology, Cornell Medical College, 515 E. 71st St., New York, N.Y. 10021.

The putative peptide neurotransmitter, substance P (SP), is present in sympathetic perikarya and nerve processes of the rat superior cervical ganglion. However, the physiologic function of SP in the sympathetic system has yet to be defined. To help determine whether the peptide plays a role in sympathetic end organ regulation, we examined SP-like immunoreactivity (subsequently termed SP) in two targets of the SCG, the iris and pineal gland. Manipulations were performed to define the source and regulation of SP in these targets. Decentralization (denervation) of the SCG, which is known to increase ganglion SP, did not alter the peptide in either target. Conversely, surgical or pharmacologic (6-hydroxydopamine) ablation of the SCG actually increased SP in both targets. Moreover, destruction of the trigeminal sensory innervation reduced iris SP to virtually blank levels. Finally, trigeminal ablation abolished the rise in iris SP subsequent to sympathetic destruction with 6-hydroxydopamine. Our observations suggest that sympathetic terminals in targets, in contrast to ganglion perikarya and processes, contain negligible quantities of SP. Consequently, SP may not be transported from ganglion to periphery, but may serve an intraganglionic function. Our studies also suggest that sympathetic terminals modulate sensory peptidergic innervation of the iris. (This work was supported by NIH grant NS 17285, American Parkinson Disease Association and Irma T. Hirschl Career Scientist Trust).

110.13 ISOLATION AND CHARACTERIZATION OF AN ASTROGLIAL MITOGENIC FACTOR. R. P. Saneto*, R. S. Morrison, and J. S. de Vellis. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

During development there are multiple factors involved in the proliferation, maturation, and maintenance of the central nervous system. Using nearly pure populations of astrocytes in culture as our assay system, we have identified and purified a mitogen from the adult rat brain that stimulates ^{3}H-thymidine incorporation into DNA. Astroglial mitogenic factor (AMF) showed no mitogenic activity in purified cultures of oligodendrocytes. Interestingly, AMF was expressed throughout the life of the rat; activity was detected in 1-2 day old rat pups through 60 day old adult rats. AMF was purified from the soluble protein fraction of whole rat brain through a combination of ion-exchange and gel filtration chromatographs. Purified AMF was demonstrated to have a molecular weight of approximately 25,000 pI of 5.4, and to be active in the presence of 2-mercaptoethanol. Mitogenic activity was sensitive to chymotrypsin, but insensitive to either trypsin, pepsin, papain, or ficin, and also sensitive to freezing. In contrast to Glial Maturation Factor, AMF did not exert morphological changes on cultured astrocytes when added to the medium for up to 48 hr.

(Research supported by the Jeanne B. Kempner Foundation, NIH and DOE).

110.14 PREFERENTIAL INNERVATION OF TRANSPLANTED INTERCOSTAL MUSCLES BY AXONS FROM DIFFERENT SEGMENTAL LEVELS. Donald J. Wigston and Joshua R. Sanes. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

We have used a novel approach to show that adult rat muscles from different segments can be reinnervated selectively. This implies that muscles possess some stable property that biases the innervation they receive. Our experiment was based on work by Purves et al., who showed that sympathetic ganglia transplanted from different levels of the sympathetic chain are reinnervated by different (though overlapping) subsets of preganglionic axons in the cervical sympathetic trunk (J. Physiol. 313: 49, 1981). Since preganglionic axons can innervate skeletal muscle, we transplanted small pieces of external intercostal muscle from either the 2nd (T2) or 8th (T8) thoracic interspace to the neck of adult rats, removed the superior cervical ganglion, and attached the proximal cut end of the sympathetic trunk to the surface of the transplant. After 2-14 weeks the innervation of transplanted muscles was assessed by intracellular recording. Muscle fibers were reinnervated, often polyneuronally, within 2 weeks. We compared the segmental origin of inputs to T2 and T8 muscles by stimulating, in turn, each ventral root contributing axons to the sympathetic trunk.

Fibers in transplanted T2 muscles were innervated more frequently by axons arising from rostral segments (T1, T2) than were fibers in T8 muscles, while T8 muscles received more inputs from caudal segments (T4, T5, T6) than did T2 muscles. This systematic difference in the segmental origin of axons innervating transplanted T2 and T8 muscles was apparent at both short (2-4 weeks) and long (10-14 weeks) times after transplantation. When 32 muscles were ranked according to their average segmental innervation, the 10 most caudally innervated transplants were T8 muscles, and 9 of the 10 most rostrally innervated muscles were from T2. The difference between T2 and T8 muscles was also evident when we visually estimated the strength of contractions evoked by stimulating individual ventral roots. Thus intercostal muscles transplanted from different thoracic segments are preferentially reinnervated by preganglionic axons arising from different levels of the spinal cord. As preganglionic axons never normally innervate muscle, the selectivity may reflect a widespread system of cellular recognition. Since the more rostral muscle (T2) received the more rostral preganglionic innervation, the selectivity may have a positional basis. If segmental identification labels do exist, further physiological and biochemical comparison of segmental muscles should provide a means of elucidating their nature and developmental role. (Supported by MDA and NSF.)

111.1 CONVERSION OF HIGH-AFFINITY BENZODIAZEPINE BINDING SITES TO LOW-AFFINITY BY PHOTOLABELING. J. W. Thomas and J. F. Tallman. Neuroscience Branch, NIMH, Bethesda, MD 20205.

Previous studies have shown that [^{3}H]-flunitrazepam forms irreversible cross links with brain tissue when exposed to UV irradiation. Comparison of the amount of [^{3}H]-flunitrazepam irreversibly incorporated and the number of benzodiazepine binding sites blocked after photolabeling has indicated that several binding sites are inactivated for each molecule of [^{3}H]-flunitrazepam incorporated. To learn the cause of this discrepancy, binding to the benzodiazepine binding sites has been examined using a radiolabeled β-carboline. Binding of the β-carboline was not altered by photolabeling; however, displacement studies revealed that photolabeling converted a homogeneous set of benzodiazepine binding sites into two subsets; one of high affinity and one of low affinity. This conversion of some binding sites to a low affinity form accounts for the discrepancy observed after photolabeling. The low affinity sites described here may be related to "cryptic" benzodiazepine binding sites present in brain that can be exposed by either physiological stress or pharmacological manipulation.

111.2 GABA PROTECTS BENZODIAZEPINE RECEPTORS FROM HEAT INACTIVATION IN THE SOLUBLE STATE. M. Gavish, Department of Pharmacology, Faculty of Medicine, Technion, Haifa, Israel.

Electrophysiological studies have shown that benzodiazepines exert their therapeutic effect by potentiation of the major inhibitory neurotransmitter in the CNS - Gamma-aminobutyric acid (GABA). Binding studies on the membrane level have shown that GABA can increase benzodiazepine binding by increasing the affinity of the benzodiazepine receptors. Previously we have shown that this is true also when the receptor is in the soluble state.

Heat inactivation studies have demonstrated that GABA can protect both membrane bound GABA and benzodiazepine receptors from heat inactivation. The benzodiazepine ligands were also shown to have a similar effect. Treatment of soluble partially purified (10 fold) benzodiazepine receptors at 55^{0}C for 30 min diminished most of the receptor binding sites. The presence of 10^{-4}M of GABA during the heat inactivation, maintained almost all of the binding sites. The relevance of this finding on the interaction between GABA and benzodiazepine receptors will be discussed.

Supported by Bat Sheva de Rotschild Foundation grant.

111.3 BENZODIAZEPINES FACILITATE THE BINDING OF GABA TO $GABA_A$ BUT NOT $GABA_B$ RECOGNITION SITES. M. D. Majewska*, D.-M. Chuang* and E. Costa. (SPON: W.J. Kinnier). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Recently it was reported that crude synaptic membranes of rat brain contain a Ca^{2+}-dependent high affinity binding site for GABA. This subtype of GABA binding sites is labeled with ^{3}H-baclofen and has been termed $GABA_B$ recognition site. THIP or isoguvacine fails to bind to $GABA_B$ recognition site and selectively work on the GABA binding site which was termed $GABA_A$ recognition site. The present study was undertaken to examine whether $GABA_A$ or $GABA_B$ sites or both are coupled to the recognition sites for benzodiazepines. Binding of ^{3}H-GABA (20 nM) to GABA recognition sites located in frozen and thawed crude synaptic membrane of rat brain was conducted at 37^{0}C in 50 mM Tris-HCl pH 7.1 in the presence of aminooxyacetic acid to block the activity of transaminases. The addition of 0.25 mM EGTA to the binding assay diminished the specific binding by about 50%; this effect could be fully reversed by the addition of Ca^{2+} (0.25-0.50 mM) to the EGTA containing mixtures. In normal conditions, diazepam (5 μM) enhanced ^{3}H-GABA binding by about 20-30%; this enhancement was increased to 60-80% when EGTA was present during binding assay. This EGTA-induced enhancement was also nullified by the addition of Ca^{2+}; at 10 mM Ca^{2+}, diazepam failed to enhance ^{3}H-GABA binding. These results suggest that EGTA changes GABA binding by preventing the binding of ^{3}H-GABA to $GABA_B$, thereby facilitating the enhancement by diazepam of the binding of ^{3}H-GABA to $GABA_A$. These EGTA effects were not found in a membrane preparation pretreated with $AgNO_3$. When binding to $GABA_A$ and $GABA_B$ was differentiated by binding ^{3}H-GABA in the presence of 40 μM of baclofen and THIP respectively, the binding to $GABA_A$ receptor was inhibited by high concentration of Ca^{2+} (>1.0 mM), but the binding to $GABA_B$ receptor was greatly stimulated by Ca^{2+}. Moreover it can be verified that ^{3}H-GABA binding to $GABA_A$ receptor was increased by diazepam but the binding to $GABA_B$ receptor was totally unaffected. In a converse experiment, we measured the enhancement of ^{3}H-flunitrazepam binding by GABA. This enhancement by GABA was virtually abolished when $GABA_A$ receptor was blocked by bicuculline but not when $GABA_B$ receptor was blocked by baclofen. Thus our results suggest that $GABA_A$ but not $GABA_B$ recognition sites are linked to the benzodiazepine recognition sites. The enhancement of benzodiazepine binding by GABA or the enhancement of GABA binding by benzodiazepines are mediated by $GABA_A$ recognition sites.

111.4 A CRITERION FOR DISTINGUISHING BENZODIAZEPINES FROM THEIR ANTAGONISTS: Effects on Benzodiazepine Enhancement of GABA Binding: B. A. Meiners* and A. I. Salama (SPON: J. Patel). Pharmacology Department, Stuart Pharmaceuticals, Division of ICI Americas, Wilmington, DE 19897.

It has been suggested that benzodiazepines facilitate GABAergic transmission and that this may be related to their clinical effects. Recently, several compounds have been discovered that bind potently to the benzodiazepine receptor but reverse the behavioral effects of the benzodiazepines. In contrast to the benzodiazepines, the affinity of these compounds for their receptor is not increased by GABA. Furthermore, it has been shown that benzodiazepines and the novel anxiolytic tracazolate (ICI 136,753) can enhance the binding of ^{3}H-GABA to frozen and thawed, Triton X-100-treated rat brain synaptic membrane fragments (Meiners, B. A. and Salama, A. I., Euro. Jour. Pharmacol., 78: 32, 1982). It was found that 3 structurally dissimilar benzodiazepine antagonists, β-carboline-3-carboxylate ethyl ester (β-CCE, 10 μM), RO15-1788 (10 μM) and CGS8216 (20 nM), had no significant effect on ^{3}H-GABA binding by themselves. In contrast diazepam (100 nM) and chlordiazepoxide (10 μM) resulted in 15% enhancement. The benzodiazepine antagonists were, however, able to reverse the enhancement of GABA binding by the anxiolytic benzodiazepines. In particular, 100 nM β-CCE completely reversed the effects of 100 nM diazepam and 1 μM reversed the effects of 10 μM chlordiazepoxide. The enhancement caused by 1 μM diazepam was reduced by RO15-1788 at 1 μM and reversed by 2 nM CGS8216. However, in sharp contrast to the benzodiazepines, the enhancement of GABA binding by tracazolate (10 μM), was not blocked by the benzodiazepine antagonists at concentrations that reversed the effects of the benzodiazepines. Thus in agreement with other data the mechanism of tracazolate appears to differ from that of the benzodiazepines.

There appears to be a parallelism between the effects of benzodiazepine antagonists on the benzodiazepine enhancement of GABA binding and their reversal of the behavioral effects of the benzodiazepines. Effects on GABA binding appear to be another criterion that distinguishes benzodiazepines from their antagonists.

111.5 THE EFFECTS OF ETAZOLATE (SQ20009) ON THE GABA RECEPTOR COMPLEX OF MAMMALIAN CORTICAL NEURONS IN CULTURE. Deborah M. Barnes, W. Frost White, Marc A. Dichter. Harvard Medical School and Children's Hospital Medical Center, Boston, MA. 02115.

The interactions of etazolate (SQ20009) with GABA-mediated inhibition and the postsynaptic GABA receptor complex were evaluated by both electrophysiological and receptor binding studies using rat cerebral cortical neurons grown in primary culture. In physiological experiments, known concentrations of drugs were applied by microperfusion to neurons which were monitored with intracellular recording. Binding assays were also performed on the intact, living neurons in culture and utilized ^{3}H-flunitrazepam (^{3}H-FLU) binding and GABA stimulation of such binding as probes of the GABA receptor complex.

Physiological analyses indicate that etazolate: (1) increases the duration of spontaneously occurring IPSPs (which have been shown to be GABA-mediated in this system)(≥0.3 uM), (2) enhances GABA-mediated hyperpolarizations and increases in chloride conductance (≥3 uM), and (3) increases chloride conductance in the absence of exogenous GABA (≥10 uM). This last effect may be due, in part, to an enhancement of the actions of endogenous GABA in the culture system, since it is reduced by inhibition of synaptic activity (with either TTX or with low Ca, high Mg medium). These effects appear specific to GABA mediated inhibition, since etazolate does not enhance glycine mediated increases in chloride conductance. They do not seem dependent on etazolate's action as a phosphodiesterase (PDE) inhibitor since a related compound (SQ20006), which is a more potent PDE inhibitor, does not enhance GABA-mediated effects.

Parallel binding studies indicate that: (1) etazolate enhances ^{3}H-FLU binding, (2) etazolate enhances the GABA-mediated increase of ^{3}H-FLU binding, and (3) GABA enhances the etazolate-induced increase in ^{3}H-FLU binding. In both the physiological and binding paradigms, bicuculline and picrotoxinin block all the etazolate effects. The etazolate effects observed in all studies show a complex dose-dependency such that low to intermediate concentrations of the drug produce increased responses and higher concentrations (between 30 and 100 uM) produce diminished responses.

We conclude that etazolate exerts its effects as a result of its interaction with the GABA receptor complex. Although the benzodiazepines also interact at the GABA receptor complex and enhance GABA effects, etazolate appears to work by a different membrane mechanism and probably acts at a different site. The effect on prolongation of IPSPs suggests that etazolate may function by increasing the "open time" of GABA-associated chloride channels. (Supported by NIH grants NS15362, NS0717 and the CHMC Mental Retardation Core Grant HD06276.)

111.6 NEW LIGANDS FOR STUDYING ACIDIC AMINO ACID RECEPTORS: [^{3}H] 2-AMINO -4-PHOSPHONOBUTYRIC ACID AND [^{3}H] L-SERINE-O-SULFATE. D.T. Monaghan*, G.E. Fagg*, E.E. Mena, M. Nieto-Sampedro, M.C. McMills*, A.R. Chamberlin*, and C.W. Cotman. (SPON: P.I. Yahr) Depts. of Psychobiology and Chemistry, Univ. of California, Irvine, Cal. 92717.

Previous studies from this laboratory have demonstrated that the potencies of the glutamate analogues 2-amino-3-phosphono-proprionic acid, 2-amino-4-phosphonobutyric acid (APB), and 2-amino-5-phosphonovaleric acid in inhibiting the glutamate-using perforant path granule cell synaptic response are similar to their potencies in the displacement of [^{3}H] L-glutamate from rat brain synaptic plasma membranes (SPMs). The L isomer of APB is the most potent in both cases. Additionally, ligand binding studies have indicated that APB is selectively interacting with a distinct sub-population of L-GLU binding sites which require the presence of Cl^- and Ca^{++} ions for binding. Other studies have indicated that L-serine-O-sulfate (L-SOS) is also selective for APB-sensitive L-GLU binding sites. Since the Cl^-/Ca^{++}-dependent, APB-sensitive L-GLU site may be a major excitatory synaptic transmitter receptor, we have developed ligand binding assays for the more selective agents [^{3}H] APB and [^{3}H] L-SOS in an effort to further characterize this binding site and evaluate its role in neurotransmission.

An APB precursor was synthesized by us and custom labelled by New England Nuclear, (26.6 Ci/mmole). [^{3}H] L-SOS was prepared from [^{3}H] serine (28 Ci/mmole). The binding of 100nM [^{3}H] APB and 10nM [^{3}H] L-SOS to Sprague Dawley rat SPMs was measured using a microfuge assay, nonspecific binding was determined with with 0.5 mM L-Glu. Both ligands exhibited L-Glu specific binding which depended upon the inclusion of Ca^{++} (2.5 mM) and Cl^- (20mM) in the assay buffer (50 mM Tris-acetate). For [^{3}H]-APB, binding was optimal at pH 6.8, 60 min, at 30°C. [^{3}H] L-SOS binding was optimal at pH 7.0, 30°C, for 20 min. [^{3}H] APB exhibited an apparent Kd of 5.0 ± 0.7 μM (S.E.M., n=5) with a Bmax of 93.1 ± 6.2 pmoles/mg protein (S.E.M., n=5). [^{3}H] L-SOS exhibited a higher affinity with a Kd of 94 ± 5 nM (S.E.M.,n=3).

The binding of [^{3}H] APB is thought to be predominately due to the L isomer since 100 μM L-APB is a more potent displacer of [^{3}H] APB binding than is the D isomer. Similarly, for both [^{3}H] APB and [^{3}H] L-SOS, the L isomers of glutamate, aspartate, and APB are more potent displacers than are the D isomers. At 100μM, ibotenic acid and quisqualic acid were potent inhibitors of binding, whereas kainic acid and n-methyl aspartic acid were poor inhibitors. Experiments are now in progress using these selective L-Glu binding site ligands to further characterize the APB sensitive glutamate binding site. (NS 08957 and AG00538)

111.7 N-ACETYL-ASPARTYL-GLUTAMATE: AN ENDOGENOUS PEPTIDE WITH AGONIST PROPERTIES AT A GLUTAMATE RECEPTOR IN BRAIN. Robert Zaczek*, Kerry Koller and J.T. Coyle. Div. of Child Psychiatry and Depts. of Psychiatry, Neuroscience and Pharmacology, Johns Hopkins School of Medicine, Baltimore, Maryland 21205.

A binding site for [^{3}H]-glutamate (GLU) has been described in the mammalian brain that appears to represent an excitatory receptor responsive to GLU. This receptor has been used as a discriminator to identify endogenous substances with potential excitatory activity. Rat brains were homogenized in ice-cold 0.4 N perchloric acid; after centrifugation, the neutralized supernatant was applied to a Dowex AG-50 column to remove primary amines including endogenous GLU. The effluent was chromatographed on a Dowex AG-1 column with a linear gradient of H_2O and 4 N formic acid. After lyophilization, the fractions were assayed for their ability to inhibit the specific binding of [^{3}H]-L-GLU (100 nM) to washed membranes prepared from the cerebral cortex. The amino acid content of the native fractions and fractions hydrolyzed in 6 N HCl at 100°C were determined by a precolumn HPLC derivatization technique.

A peak of displacement activity at 2.2 M formate, uncontaminated by free GLU, yielded GLU on acid hydrolysis. The active peptide was purified to apparent homogeneity by HPLC on a 10 μM Vydac® anion exchange column eluted with a KCl gradient. Acid hydrolysis of the active peak yielded equimolar amounts of aspartate and GLU and mass spectroscopic analysis indicated a molecular weight of 304 and a structure compatible with N-acetyl-aspartyl-glutamate (NAAG). NAAG competes at a subset of sites specifically labeled by [^{3}H]-GLU in rat cortex membrane preparation that results in a maximal 40% displacement. Scatchard plot of the NAAG displacement curve reveals mass action kinetics of inhibition with an apparent K_I of 0.42 μM. In contrast, the K_I for GLU is 1.8 μM and the K_I of quisqualic acid is 0.60 μM. NAAG did not significantly inhibit ligand-binding to GABA, kainate, dopamine or muscarinic receptors. The percent maximal displacement of NAAG at [^{3}H]-GLU sites has an uneven regional distribution in brain.

Intrahippocampal infusion of 120 nmoles of NAAG caused a prolonged seizure disorder characterized by forelimb clonus, retropulsion, rearing associated with generalized cortical seizures on EEG. The convulsant effects of NAAG were not due to the liberation of GLU since intrahippocampal injection of 250 nmoles of GLU resulted neither in behavioral nor EEG evidence of seizures. Thus, NAAG is an endogenous peptide with high affinity for a subpopulation of excitatory GLU receptors that exhibits considerable convulsant activity similar to the next most potent agonist at these receptors, quisqualic acid. (This research supported by USPHS Grants NS-13584 and MH-00125).

111.8 CELLULAR LOCALIZATION OF GLUTAMATE-SENSITIVE CEREBELLAR CYCLIC GMP: EFFECTS OF SPECIFIC LESIONS. P.J. Roberts and G.A. Foster*. Dept. of Physiol. & Pharmacol., Med. & Biol. Sci. Bldg., Univ. of Southampton, Southampton, SO9 3TU, U.K.

The cerebellar Purkinje cells are innervated by 2 major excitatory afferent systems: firstly, the mossy fibre-granule cell-parallel fibre (PF) input, and secondly, by the climbing fibres (CF's). While glutamate is a prime candidate for the parallel fibre transmitter, the identity of that of the CF's is uncertain, although it may be aspartate or glutamate.

We have demonstrated previously the stimulation of cerebellar cyclic GMP levels by excitatory amino acids, through a mechanism involving at least 3 classes of receptor, which were activated preferentially by NMDA, quisqualate, and kainate respectively. Protoveratrine, which releases transmitter substances from neuronal, but not glial compartments, also elicited a large increase in cyclic GMP, which was preventable by the quisqualate type receptor antagonist, GDEE. In the present study we have investigated the effects on the cyclic GMP response of (i) lesioning the CF input by destroying the associated cell bodies in the inferior olive, with 3-acetylpyridine (3-AP) (65 mg/kg i.p., 2 days prior to assay) (ii) depleting the granule cell population by X-irradiation (200 rads on days 8 and 9, and 150 rads on days 11, 13 and 15 postnatally) and (iii) acute destruction of cerebellar glial cells by 6-aminonicotinamide (6-AN) (10 mg/kg i.p.).

Destruction of the CF's with 3-AP resulted in an approx. 40% reduction in the maximum cyclic GMP response to protoveratrine (PTV), while the direct (postsynaptic) actions of agonists such as glu and kainate were unaffected. This reduction in response to PTV was accompanied by a similar reduction in the uptake of D-^{3}H-asp (a marker for glu/asp neurons). The Ca-dependent release of endogenous glu or asp was not altered following CF lesions. However, this may be due to any changes being obscured by a dominant PF system. Loss of PF's was similarly accompanied by a large (45%) loss in the PTV-stimulation of cyclic GMP, and by a substantial decrease in the Ca-dependent release of glu. The possible involvement of glial cells in the cyclic GMP response was investigated after 6-AN. A supersensitive response to L-glu was observed, probably due to loss of glu uptake sites/enhanced accessibility of glu to its postsynaptic receptors.

It would therefore seem that in the cerebellum, the cyclic GMP response is probably wholly accountable for in terms of the CF and PF inputs to the Purkinje cells.

Supported by the Wellcome Trust. PJR is a Nuffield Foundation Science Research Fellow, & GAF an S.E.R.C. predoctoral student.

111.9 EFFECTS OF ACIDIC EXCITATORY AMINO ACIDS ON ^{45}CALCIUM ACCUMULATION BY MOUSE STRIATAL SLICES. Konrad C. Retz and Joseph T. Coyle, Div. of Child Psychiatry, Depts. of Psychiatry and Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

The mechanisms whereby glutamic acid (GLU) and its conformationally restricted analog, kainic acid (KA) produce neurotoxicity remain unknown. Recent studies have suggested that influx of Ca^{++} ion may mediate toxicity both within and outside of the nervous system. In a previous report (Eur. J. Pharm., in press) we noted that GLU, but not KA, stimulated $^{45}Ca^{++}$ uptake in rat striatal synaptosomes. In the current studies, we have examined this process in greater detail using slices prepared from mouse striatum.

Slices, 100 microns thick, were mechanically prepared from freshly dissected mouse striata, resuspended in a Krebs-HEPES buffer (J. Biol. Chem. 252:2764, 1977), and preincubated with bubbling O_2 for 60 min. at 37°C. The rate of $^{45}Ca^{++}$ uptake in the presence of drug was measured during a 1 min period (1.0 mM $^{45}Ca^{++}$, 1 mCi/mmole), after which the reaction was terminated by addition of 3.3 vol ice-cold Ca-free buffer. The slices were then postincubated for 60 min. as described above in a Ca-free buffer containing 10 mM $LaCl_3$ (Phil. Trans. R. Soc. Lond. B 265:57, 1973), a procedure which displaces extracellularly bound Ca^{++} and entraps intracellular Ca^{++}. After this period, all buffer was removed, the tissues were dispersed into 7% TCA, and ^{45}Ca was measured by liquid scintillation spectrometry. Unless specified all studies were conducted with 4.8 mM K^+ ion present.

Preincubation in the presence of 1 mM Ca^{++} gave higher basal levels of $^{45}Ca^{++}$ uptake than were observed in slices incubated in the absence of Ca^{++}, a procedure reported to deplete the total intracellular Ca^{++} ion concentration by 50% (J. Neurochem. 10:665, 1963). However, preincubation in Ca-free buffer resulted in a greater % stimulation of ^{45}Ca by 60 mM K^+ ion and 5 mM GLU. Neither KA (to 1 mM) nor N-methyl-D,L- aspartate (to 10 mM) produced consistent effects with either incubation condition. In contrast to these results, when slices were exposed to either 1 mM KA or 5 mM GLU for the last 10 min of the preincubation period only, both drugs reduced the magnitude of ^{45}Ca uptake stimulation produced by subsequent 1 min exposure to 60 mM K^+ ion with little effect on the basal rates of uptake. Again, greater effects were observed under conditions of Ca-free preincubation. The results indicate different effects of GLU and KA on Ca^{++} disposition in brain slices and suggest that mobilization of intracellular Ca^{++} stores, rather than Ca^{++} uptake per se, may be involved in the neurotoxic effects of KA. (Supported by NS 13584, MH 00125 and the Surdna Foundation).

111.10 THE EFFECT OF INTRASTRIATALLY INJECTED KAINIC ACID ON REGIONAL BRAIN PROSTAGLANDINS. R.D. Schwarz, N.J. Uretsky, R.H. Fertel,* J.R. Bianchine. The Ohio State University, Department of Pharmacology, Colleges of Medicine and Pharmacy, Columbus, Ohio 43210.

Injection of kainic acid (K.A.) into specific brain sites has been shown to lesion neurons at the site of injection while sparing those passing through or terminating at this site. In addition, K.A. destroys neurons distal to the injection site. When injected directly into the striatum, K.A. at low doses produces abnormal movements and at higher doses convulsions. The neurochemical mechanisms producing these motor abnormalities are not currently known. We have been studying whether prostaglandins (PGs) in the brain are involved in the effects of neuroexcitatory drugs (eg. carbachol, picrotoxin) on motor function. In the present study, we have determined the effects of direct intrastiatal injection of K.A., at doses that alter motor function, on regional brain PG levels. Kainic acid was bilaterally injected into the striatum of male rats (250-300g) under halothane anesthesia using a stereotaxic apparatus. Control rats received 0.9% saline injections. The animals recovered within 1-3 min, were observed for behavioral changes and sacrificed 15 min. later by microwave irradiation. Following dissection, brain tissue was acidified with 1.0 N formic acid and exacted 3x with ethyl acetate. The amounts of PGE_2, $PGF_{2\alpha}$, 6K-$F_{1\alpha}$ and TxB_2 were then measured by radioimmunoassay. In the dose range of 0.62-2.5µg kainic acid produced characteristic abnormal motor movements. High doses produced convulsions while at lower doses explosive running was observed. The greatest changes in PG levels were seen in the hippocampus where the levels of $PGF_{2\alpha}$ and PGE_2 markedly increased. Much smaller changes were seen in the striatum and cortex. There were no signficant changes in 6K-$F_{1\alpha}$ or TxB_2 in any of the three regions examined Although pretreatment with indomethacin (20 mg/kg; Sc; 60') did not block the abnormal movements, it did block $PGF_{2\alpha}$ synthesis. In contast the increase in PGE_2 was not significantly inhibited, particularly in the hippocampus. This difference suggests that the cyclooxygenase associated with formation of $PGF_{2\alpha}$ is different from that associated with PGE_2. Although indomethacin did not block abnormal movements, the results do not exclude PGE_2 playing a causative role in the abnormal movements induced by K.A. Diazepam treatment at 2.5 mg/kg did not affect either the rise in PGs or the behavioral movements, while a dose of 30 mg/kg blocked both the motor movements and the rise in $PGF_{2\alpha}$ in the hippocampus. Thus, brain PGs are significantly altered following the intrastriatal injection of K.A. There appears to be a regional effect, since the greatest change was seen at a site distal to the striatum, the hippocampus. The ability of diazepam to block behavioral changes and PG production suggests that the excitatory effect of KA is responsible for PG production.

111.11 KAINATE-LIKE NEUROTOXICITY OF QUINOLINIC ACID IN ORGANOTYPIC CULTURES OF RAT CORTICOSTRIATAL SYSTEM. W.O. Whetsell, Jr., and Robert Schwarcz. Univ. of Tennessee Center for Health Sciences, Memphis, TN 38163, and Maryland Psychiatric Research Center, Univ. of Maryland, Baltimore, MD 21228.

Intrastriatal injections of the neuroexcitotoxic amino acid, kainic acid (KA), in the rat, have been shown to induce lesions which are morphologically and neurochemically similar to lesions observed in the brains of patients afflicted with Huntington's Disease (Kainic Acid as a Tool in Neurobiology, Raven Press,1978). Studies from our laboratories (Whetsell, Adv. in Neurol. 23:645, 1979; Whetsell, J. Neuropath. Exp. Neurol. 39:395, 1981) have demonstrated that organotypic cultures of a combination of caudate nucleus (CA) and frontal cortex (CX) from the rat are likewise susceptible to the neurotoxic effects of KA, but cultures of CA alone are not affected by KA.

Recent evidence in whole animal (rat) has indicated that an endogenous amino acid, the tryptophan metabolite, quinolinic acid (QA), is a powerful neuroexcitatory agent (Stone and Perkins, Europ. J. Pharm. 72:411, 1981). This amino acid bears certain structural analogy to KA and has thereby drawn our attention to the possibility that QA may represent an endogenous neurotoxic agent with biological activity similar to KA. Using the identical culture system in which we have studied the effects of KA, i.e., combination CA-CX cultures, we have observed that QA can induce KA-like neurotoxic effects. Although these effects can also be observed in cultures of CX alone, no neurotoxic effects from QA are observed in cultures of CA alone. The morphologic changes observed in the CA-CX cultures after exposure to QA (either 10^{-3}M or 10^{-4}M for up to 36 hours) are characterized by severe swelling and degeneration of post-synaptic elements at synapses progressing to neuronal degneration and disruption of neuropil. In control experiments using 10^{-2}M nicotinic acid, the immediate catabolic product of QA, no neurotoxic effects are observed in CA-CX or in CX or CA cultures. QA neurotoxicity in this culture system, like KA neurotoxicity, appears to depend upon some influence of CX upon CA in the cultures: the neurotoxicity may be mediated through a specificity of organization in CA when co-cultured with CX or through some other factor(s) dependent upon the presence of CX in the cultures. (Supported by USPHS Grant NS-16941).

111.12 EXCITOTOXIC CHARACTERISTICS OF INTRACEREBRAL QUINOLINIC ACID. Robert Schwarcz and William O. Whetsell Jr. Maryland Psychiatric Research Center, Univ. of Maryland, Baltimore, MD 21228 and Univ. of Tennessee, Center of Health Sciences, Memphis, TN 38163.

Neuroexcitatory amino acids of plant and fungal origin, such as kainic (KA) and ibotenic (IBO) acid, are finding increasing use as selective tools in neuroscientific research. Intracerebral application of these compounds to experimental animals causes axon-sparing lesions and, when injected into certain brain areas, can provide models for human neurodegenerative disorders.

Unilateral intrastriatal injections of >60 nmol of the neuroexcitatory (Eur. J. Pharm., 72, 411 (1981)) tryptophan metabolite quinolinic acid (QUIN) to male rats resulted in tonic-clonic movements of the contralateral forelimb, lasting approx. 4-6 hr. Upon histological examination after 4 days, nerve cell loss was noted while glia were not reduced in number. QUIN lesions extended spherically from the tip of the injection needle and increased in size in a dose-dependent manner. "Distant" neuronal necrosis, eg. in limbic structures, was never observed. Ultrastructural analysis showed prominent dendritic swelling at synaptic complexes as compared to controls. Well-preserved axons, both myelinated and unmyelinated, but only very few identifiably normal dendrites, were evident throughout the neuropil. Neurochemical measurements confirmed the axon-sparing qualities of QUIN: in a dose-dependent fashion, glutamic acid decarboxylase (GAD) and choline acetyl transferase (CAT) activities decreased, while tyrosine hydroxylase (TH) activity was not significantly altered. With respect to the contralateral, uninjected striatum, the values 4 days after 600 nmol QUIN were: GAD: 14±4%, CAT: 13±4% and TH: 87±5% (N=5). No behavioral or neurotoxic effects were observed after injection of 800 nmol nicotinic acid, the most prominent catabolite of QUIN.

Intrahippocampal injection of >60 nmol QUIN resulted in the degeneration of all local neuronal cell types, while lower doses revealed a higher vulnerability of pyramidal as compared to granule cells. In all cases, the lesions were well circumscribed and limited to the area of injection. Intrahippocampal doses in excess of 500 nmol reliably resulted in generalized convulsions.

While the morphological appearance of QUIN lesions, their potent blockade by selective IBO-antagonists (Neurosci., 7, Suppl. S188 (1982)) and the lack of pronounced convulsive properties indicate IBO-like qualities of QUIN, the preferential susceptibility of hippocampal pyramidal cells, the absence of neurotoxic effects early during development (in preparation) and the mechanisms of neurotoxicity as evaluated in tissue culture (Whetsell and Schwarcz, this meeting) appear more KA-like.

The present findings appear to justify a thorough evaluation of the function and possible dysfunction of QUIN in the CNS.

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112.1 WHAT CONTROLS INFORMATION PROCESSING IN THE LGN? Ehud Kaplan*and Robert Shapley*, The Rockefeller University, New York, N.Y., 10021. (Sponsor: James Gordon)

We have been studying the filtering of temporal and spatial information in the LGN of cats and monkeys. The spatial frequency tuning of many geniculate cells was virtually identical to that of their retinal ganglion cell inputs. Temporal tuning was sharper in most LGN cells than in the retinal input. Other cells, however, showed much less, or no temporal filtering of the input from ganglion cells.

Our recent results indicate that other brain structures, probably the cortex and reticular formation, do not merely gate the passage of information through the LGN, but also control the filtering of spatial and temporal information by LGN neurons. We recorded (with a single glass microelectrode) both the synaptic potential from a retinal ganglion cell (s-potential) and the spike fired by an LGN relay cell, in decerebrate cats, and measured the temporal and spatial transfer functions of LGN cells and s-potentials before and after IV injection of barbiturate. We found that cells which showed little or no filtering, in space and/or time, of information from the retina, showed substantial filtering of such information following IV injection of barbiturate. The degree of LGN filtering appeared correlated with the state of the EEG; a greater amount of LGN filtering was associated with synchronized slow waves in the EEG.

One possible interpretation of these results is that inhibitory interneurons in the LGN are temporally and spatially tuned, and that they are inhibited by the awake cortex via the corticofugal pathway. Disinhibition in the LGN caused by other than cortical input could also explain our results.

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112.2 THE RESPONSES OF MONKEY LATERAL GENICULATE NUCLEUS CELLS TO COLOR AND LUMINANCE CONTRAST. C.L. Colby and P.H. Schiller*. Dept. of Psychology, M.I.T., Cambridge, Mass. 02139.

This study examined the differences in the response properties of parvocellular (P) and magnocellular (M) lateral geniculate nucleus (LGN) cells in the rhesus monkey. A special focus of the investigation was the comparison of M-LGN broad-band cells with those cells in P-LGN which lack distinct color-selectivity.

The receptive fields of 135 P-LGN and 58 M-LGN cells were stimulated with a spot of light whose luminance and/or wavelength could be alternated (at 1 Hz) between two preset levels. This was accomplished with the aid of a galvanometer which exchanged the output of two independently adjustable light beams. For each cell the responses to red-green light exchange were examined at several luminance settings, thereby providing a value at which a minimal (null) response was obtained. Responses were also assessed to luminance differences at fixed wavelengths.

In the P-LGN cells lacking sharply defined color selectivity (more than 40% of our sample) were rendered unresponsive to red-green light exchange by the appropriate selection of luminance levels. Luminance settings varied over a broad range for this population of cells. Unexpectedly, the M-LGN cells could not be silenced to red-green light exchange at any setting. For the best balanced condition cells responded to each light exchange thereby yielding a response doubling. Luminance settings for the population of M-LGN cells for the best balance varied over only a narrow range. We found that the difference in the responses to the red-green light exchange between the P-LGN and M-LGN cells is in part attributable to the greater sensitivity of M-LGN cells. Our results are in agreement with those reported by Gouras (ARVO Abstr. 22:176, 1982) and by Shapley et al. (NATURE 292:543-545, 1981), and suggest that the M-LGN system is capable of detecting differences in the visual world which are due not only to luminance but also wavelength differences.

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112.3 AN INVESTIGATION OF SPATIAL RESOLUTION IN LGN X-CELLS. W. G. CHRISTEN*, G. D. MOWER* (SPON: F. H. DUFFY). DEPARTMENT OF NEUROLOGY, CHILDRENS HOSPITAL MEDICAL CENTER, BOSTON, MASS.

We assessed the separate contribution of the center and surround response mechanism to the spatial resolving ability of lateral geniculate neurons in the cat. Responses were obtained from both on- and off-center X-cells to drifting sine wave gratings. Initially a cell's resolution limit under full field stimuation was determined (no mask condition) and typically found to be 3-5 c/°. Then, the area of stimulation was confined to the receptive field center region (peripheral mask: 0.5° opening in center) or to the receptive field periphery (center mask: occlusion of the central 0.5° of the receptive field) and resolution limit was once again determined. In the center mask condition all cells showed a resolution limit below that seen in the no mask condition, however, all cells showed marked increase in resolution, typically responding to gratings of 8-10 c/°.

This increase was surprising since descriptions of center-surround organization predict that minimizing the influence of the surround should either decrease or not affect spatial resolution. There are, however, at least 2 possible explanations for this dramatic increase in resolution. In the peripheral mask condition we may be selectively stimulating the center mechanism permitting it to respond unoppossed by the surround. Alternatively we may simply be increasing the total luminance modulation by restricting the area of stimulation to a smaller portion of the receptive field center.

To distinguish between these possibilities slit masks (0.5° X 6.0°) were placed over the receptive field center and oriented parallel or perpendicular to the bars of the drifted grating. In either orientation equal amounts of center and surround are simultaneously stimulated, however, luminance modulation is maximal in the parallel orientation and minimal in the perpendicular orientation. If the increase in resolution is due to occlusion of the surround, the cells response should be independent of slit orientation. If the increase is simply due to greater luminance modulation, then resolution should be higher when the slit is oriented parallel to the grating.

The results showed the increase in resolution occured only when slit masks were oriented parallel to the grating. This suggests that the response of an X-cell to high spatial frequencies may simply reflect the sensitivity of the center to small fluctuations in mean luminance rather than to the spatial distribution of luminance over the entire receptive field.

112.4 CHANGES IN THE BALANCE AND DIFFERENCES IN LATERAL SPREAD OF Y-ON AND Y-OFF RETINAL INPUT ACROSS LAYERS A AND A_1 OF THE LATERAL GENICULATE NUCLEUS OF THE CAT. Douglas B. Bowling* and Charles R. Michael, (spon: M. Nowycky). Dept. of Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

By recording from single axons in the optic tract of the cat and injecting them with the marker enzyme horseradish peroxidase, we have examined their physiological responses and central structure. Serial reconstructions reveal the courses and destinations of the axons' collaterals, their patterns of arborization and the three-dimensional distributions of their terminal boutons.

Within layers A and A_1 of the dorsal lateral geniculate nucleus the Y-on and Y-off axons have different patterns of branching and form columns of terminal boutons that have different shapes. Both types of fibers branch extensively in the ventral portions of the layers where they form hundreds boutons. The boutons are distributed laterally (in the plane of the retinotopic map) over areas 200 to 800 microns wide. From these broad bases the columns of boutons of the Y-off axons become more narrow as they cross the layers. At the dorsal borders of the layers the terminal distributions are less than half as broad as at the bases of the columns. This gives the shape of the Y-off columns a cone-like appearance with the majority of their boutons lying ventrally in the layers.

In contrast, Y-on axons form columns of terminal boutons that are more hour-glass in shape. These columns taper from their broad bases at the ventral borders of the layers to a region of restricted lateral spread near the centers of the layers. The distributions then re-expand laterally in the dorsal portions of the layers so that the spread at the tops of the columns is about equal to that at the bases of the columns.

If Y-on and Y-off fibers from the retina are equally numerous and if the distributions of boutons visible in the light microscope reflect actual synaptic distributions, then the forms of the fibers' terminations imply two patterns of changing functional organization through the depths of the layers. First, the lateral influences of Y-on and Y-off fibers vary differentially across the layers and second, the net balance of Y-on to Y-off input varies along single projection lines across the layers.

These results are particularly interesting in light of recent reports of segregation of the ON and OFF pathways in the cat retina and in the monkey lateral geniculate nucleus.

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112.5 CORTICOGENICULATE FEEDBACK ALTERS SPATIAL TUNING IN MONKEY LGN CELLS. J. W. McClurkin* and R. T. Marrocco. Dept. of Psychology, Univ. of Oregon, Eugene, OR 97403.

We have recently reported (Marrocco et al., J. Neurosci., 1982) that a moving radial grating can activate the corticogeniculate (CG) feedback pathway in monkey. The effects are (1) not observed in the retina, (2) always reversed by cortical cooling, and (3) never observed in cells showing shift or periphery effects.

CG feedback alters the receptive field center-surround balance in a number of different ways in different cells (McClurkin et al., Soc. Neurosci. Abstr., 1981). If the receptive field center and surround mechanisms are modelled according to linear systems theory, the effects become conceptually more simple. Each mechanism acts like a low-pass filter, but peaks at different spatial frequencies. The sum of these curves represents the computed spatial tuning curve of the cell. These hypothetical curves can be described by two parameters, peak spatial tuning and bandwidth. CG feedback shifts the sensitivities of one or both mechanisms. This alters the form of the hypothetical spatial tuning curve and predicts how tuning to real sine waves will change. To test these predictions, we measured responses of LGN cells to flashing spots and drifting sine wave gratings, each restricted in area to the receptive field. We compared a cell's spatial tuning curves in the presence of the stationary radial grating with those obtained with the moving grating. The direction and magnitude of any peak shift or bandwidth change was calculated for each pair of functions. We correctly predicted the direction of the peak shift and BW changes in 75% of cells tested (n=40, 22/30 X-cells, 7/10 Y cells) and the peak shift alone in 90% of our sample.

A comparison of the sine wave data for moving vs. stationary radial grating conditions revealed the effect of feedback on spatial tuning. In 38 cells (32X, 6Y) the responses to the entire spatial frequency spectrum were significantly elevated, while in 19 cells (11X, 8Y) showed an overall reduction in response rates. Selective excitatory or inhibitory effects on the high (n=23) or low (n=12) regions of the spectrum were also observed. There was a significant tendency for the peak spatial tuning of the cell without feedback to shift toward the "middle" part of the spectrum (about 1 c/deg), to which the monkey is behaviorally most sensitive. These results show that most cells become more or less sensitive to high or low spatial frequencies, and suggest that cellular responses to edges or diffuse light are most frequently affected by feedback.

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112.6 RETROGRADE LABELLING OF NEURONS OF KNOWN BEHAVIORAL FUNCTION IN FROG TECTUM. David J. Ingle and Steven Quinn, Brandeis University, Waltham, MA 02254.

Earlier studies indicate that the frog's tectum mediates orientation towards and snapping at prey, as well as determining the direction of visually-elicited threat-avoidance behavior. We now report that the first class of "motor sequence" - turning toward prey - is dependent upon the integrity of the tectal projection to the contralateral brain stem. When this output system is severed, by splitting the tegmentum, frogs continue to snap when prey move in their lateral fields, but can only jump or hop straight forwards in response. The same animals readily turn more than 90^{0} to avoid threat, or to negotiate wide barriers.

Retrograde labelling of the contralateral pathway to the medulla with focal implants of horseradish peroxidase (HRP) has enabled us to identify the cells of origin of this pathway with a discrete visuo-motor function. Nearly all cells backfilled with HRP are located in layer 6; by contrast, a large proportion of cells filled from the ipsilateral pons are located more dorsally in layers 7 and 8, as well. The most striking characteristic of cells projecting to the contralateral medulla is that the HRP fills many horizontal dendritic arbors within the uppermost tectal layer, at the level where class-1 and class-2 fibers of Lettvin terminate. It is rare to find any dendrites of these cells branching within the class-3 recipient layer. By contrast, cells of layer 6 which are backfilled from the ipsilateral pons have dense dendritic arborization within the class-3 layer, but relatively little near the surface. From this examination, we propose that efferent cells which participate in orientation to prey have heavy (if not exclusive) inputs from the class-2 ("bug detecting") retinal fibers. These cells are probably not the "prey-sensitive" neurons which have been studies by Ewert in the toad's optic tectum (so-called T5-2 neurons) because those neurons have been shown by physiological criteria to have strong class-3 fiber inputs. We suggest that our contralateral-projecting neurons constitute one example of a putative "command neuron" (specifying turn amplitude) which is activated by a restricted class of retinal input.

112.7 INPUTS FROM VISUAL CORTEX TO DEEP LAYERS OF THE CAT'S SUPERIOR COLLICULUS: THE Y-INDIRECT PATHWAY. David M. Berson* (SPON: J.T. McIlwain) Div. Biol. & Med., Brown Univ., Providence, RI, 02912.

Retinal Y-cells are known to influence neurons in the deep layers of the cat's superior colliculus (SC) by two routes (Berson and McIlwain, J. Neurophys., '82), one direct and in part monosynaptic, the other indirect and polysynaptic. Evidence is now provided that the polysynaptic "Y-indirect" input to the deep layers, like its counterpart in the superficial SC (Hoffmann, J. Neurophys., '73), may be relayed through the visual cortex (VC).

Extracellular recordings were made under ketamine anesthesia of 201 units in the deep collicular layers (intermediate grey and below) of 7 normal cats and 7 cats with acute ablations of the ipsilateral VC, including areas 17, 18, 19, 20, 21 and the Clare-Bishop complex. Electrical stimulation was applied to the contralateral optic disk (OD), optic chiasm (OX) or ipsilateral optic tract (OT). In normal cats, 81% of deep-layer cells were driven and 59% had latency behaviors suggesting an indirect Y-cell input. In "decorticate" cats, only 30% of deep cells responded to OD, OX or OT shock and only 8% with latencies indicating indirect input from Y-cells. Among 134 cells antidromically driven from the contralateral predorsal bundle, only 3% showed Y-indirect input in decorticate cats as compared to 65% in normals. This effect is not due merely to surgical trauma, as deep units with Y-indirect input were abundant in the SC contralateral to the cortical lesion. Nor is it attributable to enhanced intertectal inhibitory influences: in a cat with chronic lesions of the contralateral SC as well as the ipsilateral VC, none of the 27 recorded deep-layer cells showed any Y-indirect influence.

In experiments in two normal, ketamine-anesthetized cats, single cathodal pulses (50 μsec, <2 mA) were delivered to area 17 through an intracortical microelectrode. Every deep-layer unit synaptically driven from the OD or OT was found to be readily excited by stimulation of the ipsilateral striate cortex (n=43). Spikes could be evoked with currents as low as 300 μA and latencies as short as 2 ms. On average, activation latencies of deep cells ($\bar{X}$=3.6 ms, range 2.0-6.8) were significantly longer than for superficial cells recorded in the same penetrations ($\bar{X}$=2.9 ms, range 1.0-7.2; n=23) and thresholds were somewhat higher, so that it is not certain that these cortical inputs to the deep SC are monosynaptic. Nonetheless, the VC clearly provides major excitatory inputs to the deep collicular layers and these are rapid enough to be compatible with their mediation of the Y-indirect influence on deep tectal cells. These results, combined with those of the decortication study, suggest that the massive polysynaptic Y-cell influence on output cells of the deep SC involves a loop through the visual cortex.

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112.8 MORPHOLOGY OF TECTO-PULVINAR NEURONS IN THE GREY SQUIRREL. P. J. May, C.-S. Lin, J. T. McIlwain and W. C. Hall, Anatomy Department, Duke University, Durham, N. C. and Division of Biology and Medicine, Brown University, Providence, R. I.

The superior colliculus is the source of a prominent pathway to the visual cortex which relays in the pulvinar complex of the dorsal thalamus. Previous studies, based on anterograde and retrograde methods, indicate that the lower portion of the superficial grey layer (SGS) projects to the pulvinar. Physiological investigation of the cat's superior colliculus show that cells in lower SGS have large fields which include up to 20% of the retinal representation as mapped onto the superior colliculus. Our anatomical studies suggest that these receptive field sizes may be related to the dendritic field sizes of the tecto-pulvinar neurons.

If the enzyme horseradish peroxidase (HRP) is injected together with a detergent (saponin) into the thalamus, tecto-thalamic neurons can be homogeneously filled with HRP reaction product. With this method approximately 70% of the lower SGS cells can be identified as tecto-pulvinar neurons. These neurons have angular somas that taper into several large dendrites. All of these dendrites branch occasionally and ascend toward the collicular surface. Consequently, these neurons possess a conical dendritic field which extends well into upper SGS and reaches at least 500μm in diameter. Cells of this type are also found in the lower SGS of Golgi-impregnated material.

Following intracellular HRP injection, we have labelled a neuron with an angular soma, and conical dendritic field. However, this cell exhibits an even larger dendritic field, which extends almost all the way to the collicular surface where individual dendrites form clusters of small terminal branches within stratum zonale and adjacent SGS. The diameter of the conical dendritic field at the collicular surface is about 4mm mediolaterally and 2mm rostrocaudally. Based on location and morphology, this neuron is presumed to be a tecto-pulvinar cell. The large size of these dendritic fields may be related to the size of the large receptive fields mapped physiologically in lower SGS tecto-thalamic neurons.

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112.9 DEMONSTRATION OF OPPONENT-COLOR PROCESSING IN MONKEY EXTRAGENICULATE PATHWAYS: THE LATERAL PULVINAR. Gary Felsten, Douglas Burman and Louis A. Benevento. P.O. Box 6998, University of Illinois College of Medicine, Chicago, Ill. 60680.

Extracellular single unit recordings were made throughout the rostral-caudal extent of the lateral pulvinar (PL) in semichronic, paralyzed, gas anesthetized macaques. Several hundred neurons were tested for responsiveness to a variety of visual stimuli and, to date, 41 of these have been characterized on the basis of their responses to chromatic stimuli. 24% of the neurons tested with colored flashes of light and moving bars of narrow to broad band-width were found to respond in a color-dependent manner. Most of these responded in a color-opponent fashion (with excitation to some colors and inhibition to others). Some neurons with clear color-opponent properties also responded with excitation or inhibition to flashes of diffuse white light, while other neurons responded with excitation to white light, but with inhibition to monochromatic lights. These properties provide the mechanisms for hue discrimination and saturation detection.

All PL neurons with color-dependent properties were binocular and had relatively large receptive fields that were spatially uniform (i.e., without any antagonistic zones). Most receptive fields were bilateral. All of the color-sensitive units responded with either tonic excitation or inhibition resembling X-like, color-opponent lateral geniculate and retinal ganglion cells. Response latencies for color-sensitive PL units ranged from 45 to 115 msec (ave.= 76.5 msec), thus falling into the short and middle latency groups, but not the long latency group, that we have observed for all PL neurons with various response properties (Neurosci. Abst. #248.4, pg.759, 1981). These latencies, taken with results from lobectomized animals and the anatomical findings on multiple midbrain inputs to PL (e.g., Neurosci. Abst. #268.4, pg. 830, 1981), indicate that the color properties may arise from midbrain relays rather than the cortex.

Consideration of these findings and patterns of afferent and efferent connections of PL leads to the conclusion that the extrageniculate visual pathways play a role in the processing of color information and suggests the possibility that the extrageniculate pathways provide color channels that are in parallel with and independent of the retino-geniculo-striate system. (supported by NIH Grant EY 2940, NIH Fellowship EY 5504 and a Grant-In-Aid of Research from Sigma Xi)

112.10 SYNTHESIS OF AN ANALYZER OF SELF-MOTION: MODELING THE ACCESSORY OPTIC SYSTEM. J.I. Simpson, R.E. Soodak* and C.S. Leonard*. Dept. Physiol. & Biophys., New York Univ. Med. Ctr. New York 10016.

On the basis of the particulars of the speed and direction selectivity of rabbit accessory optic system (AOS) neurons, we proposed that the AOS complements the vestibular system in detecting self-motion. AOS neurons, unlike the directionally selective (DS) ganglion cells that provide their primary retinal input, have a robust maintained activity (30-50 spikes/sec) in the absence of moving stimuli. Thus, AOS neurons have the necessary bias for both incremental and decremental signaling, whereas DS ganglion cells, with their virtually negligible maintained activity, do not. Here we focus on how the decrement in the maintained activity of AOS neurons arises, and offer a proposal on its functional significance. The directional tuning curves of DS ganglion cells have a cardioid-like shape (Oyster, 1968), indicating that the preferred and null directions are colinear. AOS neurons in the medial and lateral terminal nuclei typically have tuning curves showing that the best excitatory and inhibitory directions are not colinear. Such tuning curves can be constructed simply by summing a maintained activity with an excitatory and an inhibitory input represented by two DS ganglion cell tuning curves oriented so that the preferred direction of one of them corresponds to the best excitatory direction and the preferred direction of the other corresponds to the best inhibitory direction. Presumably the inhibition is provided through interposed inhibitory neurons, whose influence may reach more than one of the three AOS terminal nuclei. The non-colinearity of the best inhibitory and excitatory directions suggests that AOS neurons are involved in detecting curvilinear motion produced by rotation. Since inhibition and excitation are typically not spatially segregated within the large receptive field of rabbit AOS neurons, it seems unlikely that the significance of the non-colinearity lies in the detection of differently oriented and spatially segregated portions of arcs of rotation about a single axis. Our hypothesis is that the best excitatory direction is associated with a rotation axis different from that associated with the best inhibitory direction, and that the overall objective is ultimately to obtain maximal, bidirectional modulation for rotation about one axis and no modulation for rotation about axes in the corresponding normal plane. If so, then a preferred axis and a null plane are established, much as is the case for each of the semicircular canals. Supported by USPHS grant NS13742.

112.11 DISTRIBUTION OF DIRECTION SELECTIVITY IN THE MEDIAL, LATERAL AND DORSAL TERMINAL NUCLEI OF THE CAT ACCESSORY OPTIC SYSTEM-- K.L. Grasse and M.S. Cynader, Dept. of Psychology, Dalhousie Univ., Halifax, Nova Scotia, Canada, B3H 4J1.

Visual responses were recorded from 130 units in the nuclei of the cat accessory optic system. Using a vector analysis, the direction selective response was evaluated for neurons in the dorsal (DTN, 41 units), lateral (LTN, 46) and medial (MTN, 43) terminal nuclei. In response to a large random-dot pattern moving at a constant velocity, the DTN displayed direction selectivity primarily for temporal-nasal horizontal motion, the LTN for either vertical upward (26 of 46) or vertical downward (20 of 46) stimulus motion, and the MTN for either vertical upward (6 of 43) or downward and slightly lateral stimulus motion. E- and I-vectors for individual cells, representing the average excitatory and inhibitory directional response respectively, were usually separated by approximately 180 deg. The velocity specificity of MTN cells was not as broad as that of either DTN or LTN cells: whereas most MTN cells displayed the greatest excitatory response for stimulus velocities near 1 deg/sec, the majority of LTN cells preferred velocities anywhere from 1-13 deg/sec. While the DTN velocity specificity also ranged from around 3-13 deg/sec, there was a clear peak at 6.4 deg/sec.

In all three nuclei independent monocular testing revealed that input from the eye contralateral to the recording site was more effective than that from the ipsilateral eye in controlling the response of these units. The DTN and LTN, however, received a considerable input from the ipsilateral eye. In contrast, ipsilateral input was much less evident in MTN units. Receptive fields for cells in all accessory optic nuclei were exceptionally large and always contained the area centralis.

These findings are consistent with the notion that the accessory optic system may provide neural information concerning motion of large parts of the visual field, e.g., of the sort which is produced during eye and head movement.

This research was supported by MRC grant MT5201 and NSERC grant A9939.

112.12 2-DEOXYGLUCOSE LABELING OF THE INFRARED SENSORY SYSTEM IN THE RATTLESNAKE. E. R. Gruberg, E. A. Newman and P. H. Hartline. Biology Dept., Temple Univ., Philadelphia, PA 19122 and Eye Research Institute of Retina Foundation, Boston, MA 02114.

We have used ^{14}C-2-deoxyglucose (2DG) to identify infrared (IR) responsive nuclei in the pit viper, Crotalus viridis. Following 2DG injections into the heart, the IR sensitive pit organ was stimulated unilaterally with a periodic 0.5 sec. IR stimulus (5.6 mW/cm^2 at the pit organ) every 2.0 secs. Snakes were stimulated for 5 hours at 22°C before the brains were frozen and processed for autoradiography. The nucleus of the lateral descending trigeminal tract (LTTD, the primary IR sensory nucleus) was labeled heavily with 2DG. Labeling was bilateral, but somewhat heavier ipsilateral to the stimulated pit organ. The nucleus reticularis caloris (RC, the secondary nucleus of the IR system) was lightly labeled ipsilaterally. The middle laminae of the contralateral optic tectum (which contain IR-responsive units) were also labeled. A subcerebellar nucleus not known to be part of the IR system was heavily labeled bilaterally. No consistent labeling was found in the thalamus or telencephalon (which may indicate a limitation of our technique).

2DG labeling of the ipsilateral RC, the contralateral optic tectum, and the heavier labeling of the ipsilateral LTTD indicate that these structures are part of the IR system in the pit viper, confirming previous studies. However, the contralateral LTTD was also distinctly labeled. The LTTD does not respond to stimulation of the contralateral pit organ. Two controls were conducted. In an unstimulated snake, cutting the trigeminal nerve branches innervating the pit organ on one side eliminated 2DG labeling in the ipsilateral LTTD. Cutting the nerves to both pits eliminated all LTTD labeling. These results suggest that much of the 2DG labeling in the LTTD is due to spontaneous ongoing activity from the pit organ rather than from IR evoked activity. We confirmed this with single unit recordings from LTTD neurons. Almost all LTTD neurons had high spontaneous firing rates (10 to 18 spikes/second). When the ipsilateral pit organ was stimulated with IR stimuli of the same strength as was used in the 2DG experiments, the average firing rate was 10 to 300% greater than the average unstimulated firing rate. This increase accounts for the somewhat heavier 2DG labeling in the LTTD nucleus ipsilateral to the stimulated pit. LTTD cell activity was almost totally silenced when the afferent trigeminal nerves were cut. Our findings demonstrate that the 2DG technique can be used to identify IR-responsive nuclei in the pit viper but care must be taken in interpreting the results. Most 2DG labeling in the LTTD is due to spontaneous rather than IR-stimulated afferent activity. Supported in part by NSF grant 801539, Ortho Instruments and the Bell Laboratories.

113.1 COMPARISON OF SEROTONIN BINDING PROTEINS OF NEURONS AND ENTEROCHROMAFFIN CELLS. S. Gold*, M.D. Gershon, H. Tamir (SPON: I. Wajda). Depts. of Biochemistry, Anatomy and Cell Biology, Psych., Columbia Univ. College of P&S and N.Y. State Psych. Inst. Div. Neuroscience, New York, NY 10032.

The mucosa of the gastrointestinal tract is the largest reservoir of serotonin (5-HT) in mammals. Mucosal 5-HT is found in enterochromaffin (EC) cells, a diffuse endocrine cell that has been classified as APUD. These cells share some properties with neurons although they are entodermal derivatives. Serotonergic neurons are neurectodermal derivatives and contain a protein (SBP) that binds 5-HT specifically and with high affinity. We have found one or more proteins that bind 5-HT (Pr-5-HT) in the mucosa of the rabbit small intestine. Mucosal Pr-5-HT has been partially purified, characterized and compared with SBP obtained from serotonergic neurons of the rabbit myenteric plexus. Similarities between the two types of protein include: (i) binding of ^{3}H-5-HT is increased 20 fold by Fe^{+2}; (ii) specific binding is > 90% of total binding; (iii) binding of ^{3}H-5-HT is reduced by 70-85% by the sulfhydryl reagents, dithiothreitol (10 uM) and N-ethyl maleimide (10 uM); (iv) equimolar concentrations of 5,6-dihydroxytryptamine and 6-hydroxytryptamine reduce ^{3}H-5-HT binding by 72% and 40% respectively; (v) ^{3}H-5-HT binding is not blocked by fluoxetine (10 uM); (vi) exposure to 140mM Na^+ reduces ^{3}H-5-HT binding by 60-70%. Significant differences between mucosal Pr-5-HT and SBP include: (i) The K_D for ^{3}H-5-HT binding to mucosal Pr-5-HT was 80nM while K_D for SBP were 0.7nM and 500nM. (ii) When subjected to SDS-polyacrylamide gel electrophoresis, the complex of ^{3}H-5-HT with mucosal Pr-5-HT yields two broad peaks around 55K and 20K with much dissociation, while the complex of ^{3}H-5-HT with SBP yields a sharp peak at 45K with a small shoulder at 56K. (iii) The pH optimum for ^{3}H-5-HT binding by mucosal Pr-5-HT is 8.5 while that for SBP is 7.5. (iv) Reserpine (5uM) cannot inhibit the binding of ^{3}H-5-HT to mucosal Pr-5-HT by more than 50% while it can completely inhibit binding of ^{3}H-5-HT to SBP. The data indicate that mucosal Pr-5-HT is different from neuronal SBP; the neuronal SBP is not found in EC cells; SBP is neuron-specific, EC cells differ from neurons. Supported by grants NSF 09335, NS12969, and NS15547.

113.2 AN INVESTIGATION OF RENAL INNERVATION USING THE FLUORESCENT DYE TECHNIQUE. M.K. Donovan, B. Sripanidkulchai*, S.R. Winternitz* and J.M. Wyss. Depts. of Anatomy and Medicine, Univ. of Alabama in Birmingham.

In this study of the innervation of the rat kidney, the fluorescent dye technique was used to detect differences in the renal innervation of the right and left kidneys in both male and female rats. Adult rats (300 grams) were anesthetized with ether, and the left or right kidney was surgically exposed by a dorsal incision. An injection of a 0.5-2.0µl solution of either 2% True Blue (Illing, West Germany) or 2% Fast Blue (Illing, West Germany) was placed into the parenchyma of the kidney, and after an appropriate survival time (2-7 days) the animal was perfused transcardially. The celiac and dorsal root ganglia, splanchnic nerves, sympathetic trunks, spinal cord, and brain stem were removed from the animals. The ganglia, nerves, and trunks were placed directly on clean, uncoated slides. Frozen sections of the spinal cord and brain stem were cut and placed on clean slides. All tissues were examined using a fluorescent microscope and a Leitz A filter cube system. No differences were detected between the renal innervation of the male and female rats; however, an alteration in dorsal root ganglia (DRG) labeling was observed following right versus left kidney injections. Following left kidney dye injections, labeled cells are observed in the DRG cells located in T_8-L_2 ganglia with the greatest concentration of cells in the T_{12}-T_{13} ganglia. Following right side injections, labeled cells are present in the same segments, however peak labeling is observed one segment higher, i.e. T_{11}-T_{12}. The motor supply of the kidney is somewhat more complex than the sensory innervation. After dye injections, most labeled cells are observed in the celiac ganglia; however, some are located in the lower thoracic and upper most lumbar segments of the ipsilateral sympathetic trunk. Additional labeling is present bilaterally in the dorsal motor nucleus of the vagus and in the spinal cord nuclei associated with preganglionic sympathetic nerves. Since the celiac ganglion has been previously thought to be the major source of postganglionic fibers to the renal plexus, cell labeling in this region was anticipated. The splanchnic nerves contain a small proportion of postganglionic fibers with the cell bodies of origin in the sympathetic ganglia, thus explaining the observed labeling of a small number of cells in the sympathetic trunk. Labeled cells in the spinal cord were quite few in number and may reflect a direct innervation. Cell labeling in the dorsal motor nucleus is quite prominent and increased with larger injections. This labeling appears to be unrelated to the renal innervation, but rather appears related to blood bourne label.

113.3 HORMONAL REGULATION OF PERIPHERAL SYMPATHETIC GANGLIA. C.J. Earley*, R.W. Hamill, and L.A. Guernsey* (SPON: I. Shoulson). Neuro Res Lab, Mon Com Hosp/Univ of Roch Med Ctr., Roch, NY 14603

The effects of the hormone testosterone on neurotransmitter synthesis in peripheral sympathetic ganglia were examined in adult male Sprague-Dawley rats. Tyrosine hydroxylase (T-OH), the rate limiting enzyme in catecholamine biosynthesis was examined in the hypogastric (HG), coeliac (CG) and superior cervical ganglion (SCG) subsequent to castration. Initial studies examined HG enzyme activity since this ganglion contributes major noradrenergic terminals to pelvic sex organs. Two weeks after surgery at approximately 60 days of age, HG T-OH activity fell to approximately 30% of control. In order to decide if the observed effect was secondary to axonal injury during surgery and a retrograde reaction, animals received unilateral and bilateral castrations, and sham operation. There was no difference between sham-operated and unilaterally castrated animals, and the paired HG in the latter group had similar enzyme activity. Bilateral castration reproduced the initial observations.

In order to more clearly define the pattern of testosterone effects, HG were examined 1,2 and 4 weeks after surgery. T-OH activity was 33%, 67% and 73% of control at these three respective time points. Although castration resulted in a loss of ganglia protein, these effects were less than those observed for enzyme activity. Consequently, there was a significant reduction in enzyme specific activity. The observed alteration in T-OH activity appeared to parallel changes in the size of two target organs: vas deferens and seminal vesicles. To determine whether similar hormonal effects might occur in other peripheral sympathetic ganglia, T-OH activity was examined in SCG and CG. Enzyme activity in these ganglia was unchanged when examined one month after castration.

Enzyme activity was restored following replacement therapy with testosterone, whereas, the neural metabolite 17-B estradiol was without effect. The recovery in T-OH activity was associated with partial recovery of target organ size.

These studies suggest that hormonal factors regulate neurotransmitter synthesizing enzymes in adult sympathetic neurons and may do so via alterations in target organ size. These observations parallel similar events in the developing nervous system.

113.4 ACTIVATION OF TYROSINE HYDROXYLASE IN THE RAT SUPERIOR CERVICAL GANGLION BY NICOTINIC AND MUSCARINIC AGONISTS. J. Horwitz* and R. L. Perlman*. (SPON: R. Greenberg). Dept. of Physiol. and Biophys., Univ. of Illinois Coll. of Med., Chicago, IL 60680.

Both preganglionic nerve stimulation and carbachol nave been shown to cause an acute increase in dopa synthesis in the rat superior cervical ganglion (Ip, N. Y. et al., Abstract No. 57, American Society for Neurochemistry, 1982). The effect of carbachol was shown to be due primarily to activation of nicotinic receptors in the ganglion, but a small portion of this response appeared to be mediated by muscarinic receptors. We have found that incubation of the ganglia in the presence of either dimethylphenylpiperazinium (DMPP, 0.1 mM), a nicotinic agonist, or bethanechol (1 mM), a muscarinic agonist, results in a stable increase in tyrosine hydroxylase (TH) activity that can be detected in homogenates of these treated ganglia. Incubation of ganglia with 8-bromo cAMP (1 mM) also produces a stable activation of TH. This activation of TH occurs rapidly and can be detected after 2 to 5 minutes of incubation with these agents. The degree of activation depends upon the pH of the TH assay. At pH 6.0, the pH optimum of the enzyme, there is no difference in enzyme activity in extracts from control ganglia and ganglia treated with DMPP or 8-bromo cAMP. The activation of TH can be seen above the pH optimum and the degree of activation increases with increasing pH. At pH 6.8, in the presence of 150 uM DL-6-methyl-5,6,7,8-tetrahydropterine, TH activity in extracts of DMPP-treated ganglia is increased approximately 2-fold, and enzyme activity in extracts of 8-bromo cAMP and bethanechol-treated ganglia is increased by about 50%. Incubation of ganglia in a Ca^{2+}-free medium inhibits the activation of TH produced by DMPP, but does not block the activation due to bethanechol or 8-bromo cAMP. Although the activation of TH by DMPP appears to be Ca^{2+} dependent, the addition of Ca^{2+}, ATP and Mg^{2+} to homogenates of ganglia does not increase TH activity. Under similar conditions, cAMP does cause an activation of TH in homogenates.

In summary, we have shown that both nicotinic and muscarinic agonists activate TH in the rat superior cervical ganglion. The activation of TH by nicotinic stimulation is dependent upon extracellular Ca^{2+}. The activation due to muscarinic stimulation appears not to be dependent on extracellular Ca^{2+}.

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113.5 ACUTE REGULATION OF TYROSINE 3-MONOOXYGENASE IN THE SUPERIOR CERVICAL GANGLION IS MEDIATED BY BOTH CHOLINERGIC AND NON-CHOLINERGIC MECHANISMS. N.Y. Ip and R.E. Zigmond. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

Carbachol causes an acute increase in tyrosine 3-monooxygenase (TH) activity in the adult rat superior cervical ganglion in vitro via stimulation of both nicotinic and muscarinic receptors (Ip et al., Trans. Amer. Soc. Neurochem. 13:106, 1982). To further explore the acute regulation of TH we examined the effects of the specific nicotinic and muscarinic agonists, dimethylphenylpiperazinium (DMPP) and bethanechol. Ganglionic TH activity was determined in situ by measuring the rate of dopa accumulation in the presence of brocresine (150 μM), an inhibitor of dopa decarboxylase. Both DMPP and bethanechol caused a dose-dependent increase in TH activity with the former producing a maximal increase of 4-fold at a concentration of 0.1 mM and the latter producing a maximal increase of 2-fold at 1 mM. The effect of DMPP (0.1 mM) was abolished by hexamethonium (3 mM) but not by atropine (3 μM) while the opposite was true for the effect of bethanechol (1 mM). To determine whether this response to cholinergic agonists desensitizes, the rate of dopa synthesis was measured during a 5 min incubation with carbachol (1 mM), DMPP (0.1 mM) and bethanechol (1 mM) with and without a 15 min preincubation with these compounds. Preincubation did not affect the magnitude of the response to carbachol or bethanechol but reduced the response to DMPP by 60%. Substance P (1-10 μM) had no effect on TH activity by itself, but it inhibited the stimulatory effect of DMPP. A maximum inhibition of about 70% was found with substance P at a concentration of 10 μM. Substance P did not affect the response to bethanechol. In addition to the nicotinic and muscarinic stimulation of TH, experiments with preganglionic nerve stimulation suggest the existence of a third, noncholinergic component in the regulation of this enzyme. Preganglionic nerve stimulation at 10 Hz for 30 min produced a 6-fold increase in dopa synthesis. Hexamethonium (3 mM) and atropine (3 μM) only reduced the magnitude of this increase by 32%. Stimulation at 10 Hz for 1 out of every 6 sec for 30 min resulted in a smaller (2-fold) increase in dopa synthesis which was completely unaffected by hexamethonium and atropine. Under both stimulation conditions, physostigmine (10 μM) increased the magnitude of the TH response and these effects of physostigmine could be totally blocked by hexamethonium and atropine. Physostigmine did not affect the magnitude of the hexamethonium and atropine resistant response. We conclude that preganglionic nerve stimulation releases a second transmitter, in addition to acetylcholine, which leads to a stimulation of TH activity and that the relative importance of the cholinergic and non-cholinergic components depends on the parameters of stimulation. Supported by USPHS Grants NS 12651 and MH 00162.

113.6 LONG TERM REGULATION OF TYROSINE HYDROXYLASE BY DOPAMINE IN CULTURED ADRENAL MEDULLAE. W. J. Burke and T. H. Joh. St. Louis Univ. Sch. of Med., St. Louis, MO 63104 and Cornell Univ. Med. Coll., New York, NY 10021.

We sought to determine whether the long term regulation of tyrosine hydroxylase (T.H.) could be altered by its product dopamine (DA). Explants of rat adrenal medullae were cultured for 20 hr. in the presence or absence of DA in defined medium. There was a 78% decrease in T.H. activity in homogenates of tissue cultured in the presence of 1 mM DA. The decrease in T.H. was proportional to the concentration of DA in the medium. The decrease was not due to a direct effect of catecholamines (CA) on T.H. since the total CA content of DA treated tissue was decreased by 60%. Dihydroxyphenylalanine (DOPA) produced a similar 74% decrease in T.H. when added to the medium at a 1 mM level. This decrease, however, was prevented by addition of 3-OH Benxylhydrazine to the medium to block conversion of DOPA to DA. The methylated and oxidative metabolites of DA and CA produced no decrease in T.H. Two other medullary enzymes, monoamine oxidase and acid phosphatase, were not affected by DA in the medium. We conclude that cytoplasmic levels of DA, but not its metabolites, regulate T.H. thru long term mechanisms.

113.7 LOCALIZATION OF EPINEPHRINE, NOREPINEPHRINE AND METHIONINE-ENKEPHALIN IMMUNOREACTIVITY IN THE RAT ADRENAL MEDULLA. R. Elde, V. Holets, R. Ho and F. DiTirro. Depts. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455 and The Ohio State Univ., School of Medicine, Columbus, OH 43210.

Methionine-enkephalin (ME) has been shown to be released from the chromaffin cells of the adrenal medulla, and to be co-contained with epinephrine (EP) and not norepinephrine (NE) in the bovine adrenal gland. Using the induced fluorescence of NE in the rat adrenal medulla, combined with the indirect immunofluorescent localization of EP, NE and ME immunoreactivity, we have investigated the distribution of ME-containing chromaffin cells in the rat adrenal medulla.

The EP and NE antisera were generated in rabbits by immunization with an epinephrine- or norepinephrine-methylated bovine serum albumin complex. The rats were perfused with buffered 4% paraformaldehyde-0.2% glutaraldehyde. Cryostat sections (10 um) were directly observed with a Zeiss fluorescence microscope (transmitted UV illumination; 355-425/455 nm) to identify the NE containing chromaffin cells. The sections were subsequently rinsed in phosphate buffered saline, and adjacent sections were incubated with either EP, NE or ME antisera. Additional sections were incubated with antisera which had been preabsorbed with EP, NE or dopamine (EP and NE antisera) and ME (ME antisera). The sections were observed with a Zeiss fluorescence microscope (epifluorescence; 390-490/510 nm).

The NE immunoreactivity was found in the same chromaffin cells as were observed to fluoresce using the induced catecholamine fluorescence of NE. The EP and ME immunoreactivity was localized in the NE negative chromaffin cells. In serial sections, the ME immunoreactive chromaffin cells corresponded to the EP immunoreactive cells, and not the NE immunoreactive cells. EP and NE immunoreactivity was not diminished by the preabsorption of the antisera with NE or dopamine (EP antisera) and EP or dopamine (NE antisera). Preabsorption of the EP, NE and ME antisera with the respective antigens (EP, NE and ME) blocked the staining of the chromaffin cells.

The findings of the present study are consistent with the findings in the bovine adrenal medulla. It has been suggested that the endogenous opioid peptides are released with epinephrine and norepinephrine from the chromaffin cells, and serve to modulate the basal secretion of the catecholamines. The opioid peptides in the rat adrenal medulla appear to be co-contained with EP, and may be serving this function in the rat adrenal medulla.

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113.8 CYTOCHEMISTRY OF THE ADRENAL CHROMAFFIN CELL: LOCALIZATION OF ADENYLATE CYCLASE AND ACETYLCHOLINESTERASE. Stephen W. Carmichael, Department of Anatomy, West Virginia Univ. Sch. of Med., Morgantown, WV 26506

Biochemical studies have given conflicting information as to the distribution of two enzymes in the adrenal chromaffin cell. There is disagreement on the presence of adenylate cyclase and acetylcholinesterase on the chromaffin vesicle membrane or within the vesicle. Wilson and Kirshner (Mol. Pharmacol. 13:382, 1977) found that neither enzyme co-localized with markers for chromaffin vesicles. However, Zinder et al. (Biochem. Biophys. Res. Comm. 79:707, 1977) found a low but measurable amount of adenylate cyclase activity co-localized with vesicle membrane markers. Also, Gratzl et al. (Biochim. Biophys. Acta 649:355, 1981) found acetylcholinesterase both on the membrane and in the soluble contents of vesicles. Although the conflicting results could possibly be explained by differences in the methodologies, the presence of the enzymes in/on the vesicle is not established. Cytochemical studies were done to help clarify the situation. Modification of the method of Howell and Whitfield (J. Histochem. Cytochem. 20:873, 1972) has clearly localized adenylate cyclase activity on the plasma membrane of unstained, briefly osmicated specimens from cow and hamster. Faint lead precipitates are sometimes seen in patches of the vesicle membrane. This would support the existence of a low level of enzyme activity. Modifications of the method of Somogyi et al. (Proc. R. Soc. Lond. Biol. 191: 271, 1975) were used to localize acetylcholinesterase activity. Experiments with glutaraldehyde-fixed adrenal medulla from cow, pig, and hamster have localized enzyme activity extracellularly (neuronal structures), on the plasma membrane, on ER, and on the nuclear envelope, but not on or in chromaffin vesicles. This is in agreement with Somogyi et al. (1975) and other studies. In additional experiments, slices of fresh adrenal medulla were cut at 100 μm on a Vibratome® and incubated for up to 60 min prior to fixation. Although structural integrity was compromised, faint precipitates could be visualized at the chromaffin vesicle in unosmicated unstained specimens. The precipitate is more distinct at the membrane than in the vesicle. This result is interpreted to indicate either a low level of acetylcholinesterase activity at the vesicle membrane with a lower activity inside the vesicle, and/or the enzyme is sensitive to glutaraldehyde.

This work was supported in part by grants from the West Virginia Affiliate of the American Heart Association, WVU Medical Corporation and NIH Biomedical Research Grant 5 507-RR05433-18. Suggestions by Istvan Benedeczky and the assistance of Diane Ulrich and Lois E. Williams are gratefully acknowledged.

114.1 FACIAL AND VIBRISSAE MOVEMENTS PRODUCED BY STIMULATION OF AREA 5a IN THE CAT. R.S. Waters and H. Asanuma. The Rockefeller University, New York, NY 10021.

We previously reported (Favorov et al., Neurosci. Abstr. 6:124) that neurons lying along the posterior bank of the ansate sulcus in areas 5a and 5b project topographically to the motor cortex, area 4γ. Neurons in the lateral branch of this ansate region, respond, in large part, to natural stimulation of the forelimb and face region, with the face representation located in the more lateral part of the ansate region. In the present study, we delivered intra-cortical-microstimulation (ICMS) to the face region of area 5a and produced motor effects to the facial musculature and/or vibrissae with threshold currents between 4-30uA.

Subjects were anesthetized with halothane gas and a chamber was installed over the sensory and parietal cortices. Following surgery, the subjects were sedated with ketamine (1-2mg/kg) throughout the experiment. A tungsten-in-glass microelectrode was inserted in the posterior bank of the ansate sulcus and the face region was identified by examining the receptive fields of neurons using natural stimulation (e.g., touch and pressure to skin). Trains of 10-12 cathodal pulses (0.2ms duration, 300Hz) were then delivered along the penetration through the same electrode and the motor effects were examined.

A total of 177 locations were found where stimulating currents of 30uA or less produced movement of the vibrissae and/or the muscles of the face. Of this total, 45% of the sites were activated with currents between 20-10uA and 33% of the sites were activated with less than 10uA. At threshold stimulation both single vibrissa and clusters of vibrissae could be activated, while motor effects to the face were generally found in a restricted location on the face. In general, a close coupling was found between the sensory input and motor output.

Since low threshold loci for motor effects of the facial musculature are known to exist in the anterior sigmoid gyrus (ASG) of the motor cortex and area 2pri of the second somatosensory system and since these areas have interconnections with one another, the effect we described might be mediated through these other cortical regions. To examine this possibility we ablated the ASG and area 2pri and report that the integrity of these regions is not necessary for the low threshold effects from the posterior ansate region. These results suggest the existence of an input-output coupling in the parietal cortex not unlike that reported for the motor cortex.

114.2 CHARACTERISTICS OF PROJECTIONS TO MOTOR CORTICAL NEURONS FROM VL AND AREA 2 OF SENSORY CORTEX AS STUDIED BY INTRACELLULAR RECORDING. E. Kosar, R.S. Waters, N. Tsukahara and H. Asanuma. The Rockefeller University, New York, N.Y. 10021

The interests of our laboratory are currently focused on the plasticity of connections within the motor cortex. We have initiated the study by examining the mode of synaptic terminations of the various inputs on to motor cortical neurons.

In the anesthetized cat (Nembutal 30 mg/kg) a double closed chamber was installed over the motor and somatosensory cortices. Stimulating electrodes were implanted in the pyramidal tract (PT), VL and in area 2 of S-Cx. Surface evoked potentials were recorded at the onset of the experiment to insure the proper functioning of all implanted stimulating electrodes and to localized the cortical foci showing maximal activation from each of these electrodes. The stimulating current for the VL and PT electrodes was 1.5 times the strength for the maximum evoked potential and 30μamp for the cortical electrodes. Intracellular recording of motor cortical neurons was then initiated. Our results indicate the qualitative differences exist in the location of neurons activated by VL and those activated by area 2 stimulation. All neurons responding with monosynaptic EPSPs to cortico-cortical stimulation were located in lamina III whereas the VL-activated cortical neurons were widely dispersed throughout the depths of the motor cortex. Intracellular injection of HRP into a cortico-cortical neuron resulted in the labeling of a spiny, stellate cell within lamina III. Differences between the 2 populations of cells were also noted with respect to their latency. Neurons activated from area 2 had a mean latency of 2.12 ± .03 msec whereas VL-activated neurons were characterized by a shorter latency (1.47 ± .01 msec). The time to peak was measured for the EPSPs but no significant differences existed between these 2 populations of cells. In general, individual motor cortical neurons were activated by only one input. No cortico-cortically excited neurons responded to pyramidal tract stimulation, although several neurons were found which showed activation from both VL and PT. Only a few cells were identified as receiving input from VL and area 2 combined and these were situated at the lamina II-III border.

In conclusion, qualitative differences were observed in the laminar site of termination of different inputs within the motor cortex. VL and area 2 project discretely to differing populations of motor cortical neurons with little convergence.

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114.3 ROLE OF PERIPHERAL SENSATION DURING SKILLED MOVEMENT IN THE MONKEY. H. Asanuma and K. Arissian*. The Rockefeller University, New York, N. Y. 10021.

The functional role of sensory input to the motor cortex during voluntary movement was examined by severing the sensory input pathways. The motor cortex receives peripheral information relayed through the somatic sensory cortex. In addition, the motor cortex receives peripheral input directly from the thalamus (Asanuma et al., 1979) and the input comes through the dorsal column (Asanuma et al., 1981).

Monkeys were trained to pick up a pellet of food from a food well on a rotating board. After reaching criterion performance, the effect of sensory cortex ablation was studied in two monkeys (Macca facicularis). Following recovery from surgery the monkeys appeared normal in the cage, but examination of hand skill by rotating board revealed a degree of clumsiness in using the contralateral hand. However, the hand skill recovered near to preoperational level within a week. The effect of dorsal column section was examined in 3 monkeys, one without and two with prior sensory cortex ablation. The monkey without prior S-Cx ablation could stand and walk normally in the cage immediately after the operation. Examination of the hand skill by the rotating board revealed that dorsal column section produced severer motor deficit than sensory cortex ablation. However, the subject recovered from the deficit within 2 weeks. In the other two subjects, the hand skill was examined using the hand ipsilateral to the ablated sensory cortex and the results were similar.

Combination of sensory cortex ablation and dorsal column section produced severe motor deficit. After recovery from the operation, they could stand in the cage cautiously, but could not walk. The hand contralateral to the lesioned cortex was severely paralyzed and did not recover during the period of observation which lasted 2 months.

The results are interpreted in the following way. After ablation of the sensory cortex, the motor cortex still received sensory input directly from the thalamus. Following dorsal column section, the motor cortex received peripheral information via sensory cortex which received peripheral input through the spino-thalamic tract. Elimination of total peripheral information to the motor cortex thus produced severe motor deficit. (Supported by NIH grant NS-10705)

114.4 ORGANIZATION OF MUSCLE AND OF MOVEMENT CONTROL ZONES IN THE ARM AREA OF PRIMATE MOTOR CORTEX. D.R. Humphrey, D.J. Reed & A.M. Mitz*, Lab. Neurophysiol., Emory Sch. Med., Atlanta, GA 30322.

Previous studies have suggested that the outflow from the primate motor cortex is organized for the control of single muscles, or small sets of muscles acting at single joints. If so, however, it is not clear how the patterns of synergistic activity that are necessary for many voluntary movements are generated. Even apparently simple movements about a single joint, for example, may involve a complex pattern of joint-moving and joint-stabilizing activity by several muscles, acting about the same and even adjacent joints. A question of major importance is the extent to which such patterned activities depend upon the intrinsic efferent organization of the motor cortex. Are they determined principally, for example, by the patterns of afferent input to separate single muscle control zones in the precentral gyrus? Or, does each subregion of the gyrus contain an intermingling of muscle control zones, so that a spatially confined afferent input might evoke a patterned, functional output? To pursue this question further, we used intracortical microstimulation and chronic EMG recording techniques to map out specific movement and muscle control zones within the precentral arm area of the lightly tranquilized monkey. Some 800-1,000 points were stimulated along a total of 90-150 microelectrode tracks within the precentral gyrus of each of two monkeys, using a 10-pulse train (0.2 msec pulse, 300 pps) and current intensities of 2-40 μA. Threshold-contour plotting methods were used to outline the spatial extent of zones which evoked simple movements about the fingers, wrist, elbow or shoulder, and those evoking EMG activity in wrist flexor-extensor, biceps, triceps and deltoid muscles. Our results are as follows. (1) EMG activity could be evoked in a particular limb muscle from multiple foci. Within some of these foci, stimuli of low to moderate intensity (5-20 μA) evoked simple movements about the joint or joints spanned by that muscle. Within the remaining foci, the muscle was activated as a stabilizer during different movements occurring at the same or adjacent joints. (2) Conversely, stimulation at moderate intensities within a single subregion often evoked an organized movement, involving activity in several muscles. (3) Preliminary unit recordings from several of the foci for the wrist extensor muscles provide consistent data: cells in some foci fired only during wrist extension, those in other foci only when the wrist extensors acted as stabilizers during another movement. We propose, therefore, that the outflow from particular, circumscribed regions of the primate motor cortex may be organized for the production of specific, fundamental movement patterns. (Supported by NIH Grant NS-10183).

114.5 MOTOR CORTEX RESPONSES TO ARREST OF ACTIVE MOVEMENT.
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The present study examined the effects of afferent inputs occurring in the course of active movement, where the afferent inputs were generated by stopping the movement. The purpose of the experiment was to provide information as to motor cortex events occurring in relation to a "mismatch" between intended and actual movement.

A visual tracking paradigm was used to train monkeys to maintain a stable position and then to flex or extend the elbow by about 10°. In approximately half the trials, movements were stopped by a servo-controlled torque motor. The time course and magnitude of motor cortex discharge was compared for stopped and unstopped movements.

It is well known that arrest of movement tends to exaggerate those patterns of muscle discharge that would have occurred with unstopped movements. Thus, for a muscle such as biceps that discharges with flexion, arrest of the flexion movement causes a prolongation and enhancement of biceps activity, whereas arrest of extension causes a prolongation of biceps silence. Using these stop-effects in muscle as a model, effects of stop on motor cortex neurons were classified according to whether they were LM (like muscle, with discharge exaggerated by stop) or UM (unlike muscle, with discharge diminished by stop). Analysis of results to be presented in this abstract is restricted to neurons having a clear relationship to active movement and in addition showing a modification of activity when movement was arrested.

Both LM and UM stop-effects were observed in motor cortex, and there was a highly significant relation between the lead time of unit activity prior to onset of movement and the type of stop-effect (LM or UM) that the unit exhibited. Units were divided into two categories according to the temporal relationship of their discharge with respect to onset of active movement (before and after median onset time) and the four-fold table resulting from these categorizations is shown below:

	Early	Late
Like Muscle	67	29
Unlike Muscle	1	39

It is apparent that LM stop-effects (consistent with the idea of a transcortical servo) occur in almost all motor cortex units that discharge early in relation to active movement. In contrast, the UM stop-effects that are predominant in units with later onset times are what one would predict for neurons being driven by signals prevented by the stop (e.g., information as to joint position).

114.6 THE ORGANIZATION OF THE PRIMARY MOTOR CORTEX CONTROLLING LARYNX, TONGUE, JAW, AND FACE IN THE MONKEY.
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In a series of studies on chronic rhesus monkeys, the nature of representation of laryngeal, tongue, jaw, and face muscles on the primary motor strip lateral to the subcentral dimple was investigated using the microstimulation technique. Each monkey was first implanted with a microelectrode chamber. In a subsequent session, bipolar pairs of EMG electrodes (fashioned as a prefabricated array) were implanted in eleven laryngeal muscles to monitor responses during cortical microstimulation. Face, tongue, and jaw responses were observed visually and documented by videotaping. Following implantation, physiological sessions were conducted on an animal under ketamine anesthesia every 2 to 3 days for 3-4 months. During a session, an epoxylite coated tungsten microelectrode with 25u exposed tip was hydraulically advanced into the cortex orthogonal to its surface. Pulse trains of 100 msec, 333/sec, 200 usec pulse width, and 25 uamps were delivered with the microelectrode. When responses were observed at a site, the stimulus current was lowered to determine the threshold for each response.

Several conclusions can be drawn from these studies. First, with regard to the macro-organization, the laryngeal muscles had a significant representation but less extensive than previously reported using surface macrostimulation (Hast, B.R. 73:229:1974). Their representation was largely limited to a strip extending from the subcentral dimple laterally to the sylvian fissure. Tongue and jaw muscles also had a significant representation, overlapping the laryngeal strip and extending medially. Face muscles were weakly represented by a zone running posterior-medial to anterior-lateral and included those muscles clustering about the corner of the mouth. The zone is apparently the lateral extension of the anterior face region described previously by McGuiness (J.Comp.Neurol. 193:591:1980). Second, the muscle with the most prominent representation in this region of cortex was the posterior-cricoarytenoid (PCA, the glottis opener). Excitation of a laryngeal adductor or strap muscle was always associated with inhibition of the PCA. PCA inhibition was also observed during microstimulation at sites outside the laryngeal strip. This region of cortex evidently has a strong influence in preventing glottal opening presumably to prepare for vocalization. Finally, the majority of responses observed during low current stimulation (less than 10 uamps) involved more than one muscle. At least 2 muscles responded during stimulation within the laryngeal strip, often with indistinguishable thresholds. Tongue and jaw responses were also commonly observed in association.

114.7 MODULATION OF THE JAW OPENING REFLEX DURING FICTIVE MASTICATION IN THE RABBIT.
J.P. Lund, Université de Montréal, S. Enomoto*, K. Hiraba*, H. Hayashi*, M. Katoh*, Y. Nakamura*, Y. Sahara* and M. Taira*, Tokyo Medical Dental University.

Strong mechanical or noxious stimulation of the face or mouth causes a short latency digastric muscle jaw opening reflex response in most animals, and it has previously been shown in rabbits (Lund, Rossignol and Murakami, Can. J. Physiol. Pharmacol. 59: 683-690, 1981) that the amplitude of this response varies during the masticatory cycle. When strong (presumably noxious) electrical stimuli were used, the amplitude was highest during the jaw closing phase of mastication when the digastric muscle is inactive, and lowest when the muscle is active in the jaw opening phase. Thus it was concluded that the gain of the reflex response to noxious stimuli is controlled by premotoneuronal mechanisms. The present experiments were designed to test the hypothesis that these mechanisms do not depend on sensory feedback.

The experiments were performed on rabbits anesthetized with urethan. Mastication was evoked by repetitive stimulation of the motor cortex through a concentric bipolar electrode, and wire electrodes were implanted in the upper lip to evoke the jaw opening reflex. The digastric nerve was dissected on one side and the neurogram recorded with wire hook or sleeve electrodes. The animals were paralysed with gallamine triethiodide and artificially respirated. The masticatory cycle was measured from the rhythmical bursts in the digastric neurogram, which normally occur during the jaw opening phase of mastication. The amplitude of the reflex responses to lip stimulation during fictive mastication were compared to preceding control responses. The results were very similar to those previously obtained in unparalysed rabbits. Firstly, the mean amplitude of the reflex response was below mean control levels during mastication. However, the responses that occurred in the phase that would correspond to late jaw closure in the normal state equalled or exceeded the control level.

It is concluded that the jaw opening reflex arc can be controlled during mastication by central mechanisms. Cyclical variations in sensory feedback are not essential for either the reduction in reflex gain during the phase of the digastric muscle activity or its increase in late jaw closure.

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114.8 DIVERGENT SYSTEMS ORIGINATING FROM THE DORSAL COLUMN NUCLEI IN CAT.
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The most well known efferent projection of the dorsal column nuclei (DCN) involves DCN's dense and well organized pathway through the medial lemniscus to the ventrobasal complex of the thalamus (VB). This pathway is part of a more comprehensive DCN-VB-sensorimotor cortex circuit. Neurons in DCN, however, also project to regions other than VB. These targets include specific portions of the zona incerta, posterior group, red nucleus, pretectum, tectum, cerebellum, inferior olive and spinal cord.

In the course of investigating details of DCN's efferent connectivity using a number of different orthograde, retrograde, and bidirectional tracers in various combinations, it was found that the DCN neurons projecting to the pretectum and tectum were different from those projecting to VB (Bull, Mitchell and Berkley, Soc. Neurosci. Abstr., 1981, 7, 394). These differences suggested that DCN provided components for multiple and relatively independent somatic sensory pathways. In support of this suggestion, it was found that the DCN-recipient parts of the pretectum were themselves specifically connected with the DCN-recipient parts of the inferior olive (Mitchell, Bull and Berkley, Soc. Neurosci. Abstr., 1981, 7, 395).

The purpose of the present investigation was to examine the somatic sensory-related connections of the pretectum in further detail. Using ^{3}H-leucine, ^{3}H-acetylWGA, ^{3}H-apoHRP, HRP or WGA:HRP, it was found that neurons located in the DCN-recipient portions of the pretectum projected to the DCN-recipient portions of the tectum and zona incerta (as well as the inferior olive), but not to VB, the red nucleus, or the posterior group. In addition, neurons located in the DCN-recipient portions of the zona incerta (and possibly the tectum) projected back to the pretectum.

These results indicate that DCN is involved in at least two systems. One system consists of the classic DCN-VB-cortex circuit. Another system consists of a complex circuit of interconnections between the DCN, inferior olive, pretectum, tectum and zona incerta. Taking these results together with those indicating that different populations of DCN neurons are involved in these two systems, it seems reasonable to suggest that the systems are relatively independent of each other and that they serve different roles in somatic sensation.

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115.1 THE ROLE OF A PERSISTENT INWARD CURRENT ACTIVATED DURING RHYTHMIC FIRING IN CAT LUMBAR MOTONEURONS. P.C. Schwindt* and W.E. Crill (SPON: H.D. Patton). VA Medical Center & Depts of Physiology & Biophysics, and Medicine, University of Washington, Seattle, WA 98195.

Cat lumbar motoneurons were impaled with two microelectrodes, and then rhythmic firing properties were determined by intracellular injection of constant current pulses. Membrane currents activated by steps in the same voltage range as the membrane potential between spikes (the "pacemaker potential") were subsequently determined in the same neurons using the technique of somatic voltage clamp. Such subthreshold voltage steps activated two previously described ionic currents, the inward calcium current (I_i) and the slow outward potassium current (I_{Ks}). At fast firing rates, the pacemaker potential remains entirely within the range of voltages over which I_i is tonically activated during voltage clamp. At slower firing rates the pacemaker potential only partially enters the voltage range where I_i is activated. Cells displaying a strong, tonic, inward current developed a secondary range during steady firing, while cells displaying a weaker inward current maintained a linear f-I curve. A mixture of I_{Ks} with I_i is particularly marked in the latter cells. After I_i deteriorated due to impalement injury, the cells did not fire at fast rates; firing rate plateaued at higher injected currents. The use of two independent intracellular microelectrodes allowed accurate measurement of the somatic voltage (firing level) at which spikes are initiated. Firing level (FL) increases 50-150% when the cells are driven from minimum to maximum firing rate. It is the further depolarization caused by the rise in FL that ensures I_i activation. The FL increase is caused by the accommodative properties of the initial segment. Except at the fastest firing rates, FL occurs at less depolarized voltages than those which activate somatic sodium current. Motoneurons with normal firing properties showed minimal sodium inactivation over the voltage range traversed by the pacemaker potentials at slower firing rates and about 50% inactivation during the maximum firing rate. Both the progressive rise of FL and the subthreshold activation of I_{Ks} tend to decrease the slope of the f-I curve. We propose (1) that the basic role of I_i is to aid in maintaining a linear f-I curve by means of its depolarizing action, particularly at fast firing rates, and, (2) in cases where I_i is particularly strongly activated relative to I_{Ks}, it leads to the development of a secondary range during steady firing.

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115.2 THE DENDRITES AND SOMATA OF HIPPOCAMPAL PYRAMIDAL CELLS GENERATE DIFFERENT ACTION POTENTIAL PATTERNS. Robert K.S. Wong and Roger D. Traub. Dept. of Physiol. and Biophysics, U.T.M.B., Galveston, TX 77550 and IBM Watson Research Center, Yorktown Heights, NY 10598

Intradendritic records from distal apical dendrites of the CA1 pyramidal cell showed that burst firing was generated at these sites. In contrast, the somata of the same group of cells usually produce repetitive firing pattern both spontaneously and in response to direct depolarization. To further examine the possibility that bursts are generated in the dendrites and not in the somata of these cells, we have applied extracellular stimulation to directly excite the dendrites of these pyramidal cells. Intrasomatic recordings showed that upon depolarization of the dendritic elements by the extracellular stimulating electrode, bursts of action potentials were elicited in the soma. When hyperpolarizing current was injected into the soma during dendritic stimulation, the full size action potentials were blocked and revealed the underlying bursts of spikelets (10-15mV). When sufficient hyperpolarization was applied, the spikelets were also suppressed. Direct depolarization of the soma, however, produced repetitive firing. We have carried out additional experiments to obtain simultaneous intracellular recordings in the somatic and distal dendritic region. On two occasions we obtained electrotonically coupled recordings from the two regions and showed that the element in the dendritic region exhibit bursting activity while that in the somatic region showed repetitive firing. These recordings could have been obtained from the same pyramidal cell or from electrotonically coupled cells. Regardless, the results support the hypothesis that the dendrites and somata of the CA1 pyramidal cell exhibit different firing patterns. Dendritic burst firing has an obvious role in determining the spontaneous output pattern of the cell. Furthermore, the burst also contribute to potentiate the efficacy of excitatory synaptic potential. (Supported by NS18464 and Klingenstein *Foundation*).

115.3 PROPERTIES OF THE LATE HYPERPOLARIZING POTENTIAL IN HIPPOCAMPAL PYRAMIDAL CELLS IN VITRO. N. R. Newberry* and R. A. Nicoll, Depts. of Pharmacology and Physiology, University of California, San Francisco, CA 94143.

A synaptically-evoked late hyperpolarizing potential (LHP), in CA1 hippocampal pyramidal cells in vitro, has recently been described (Nicoll and Alger, Science 212: 957, 1981). This LHP is due to an increase in G_{K^+} and is resistant to GABA antagonists. It was suggested that the LHP may represent a Ca^{++}-activated G_{K^+} ($G_{K(Ca)}$) induced by the excitatory transmitter. Although intrasomal injection of the Ca^{++} chelator, EGTA, reduced the LHP in a few cells, in the majority of cells it was resistant to these injections (Alger and Nicoll, unpublished observations). This raised the possibility that the LHP was due either to a remote dendritic $G_{K(Ca)}$ or that another mechanism was involved in its generation. Consequently we searched for an agent which would block $G_{K(Ca)}$ and that could be bath applied so as to reach the entire cell surface.

An intrasomal depolarizing current pulse, which induces a train of action potentials, evokes an afterhyperpolarization (AHP) which is due to $G_{K(Ca)}$. This AHP was used to monitor the $G_{K(Ca)}$ in the cell. Bath application of the cyclic-AMP analogue, 8-bromoadenosine 3',5'-cyclic monophosphate (1mM), abolished the the direct AHP (see Madison and Nicoll, this meeting) but it did not depress the concomitantly monitored LHP. This indicates that $G_{K(Ca)}$ is not primarily involved in the LHP. It is therefore possible that the LHP is a slow non-GABA mediated IPSP. The enkephalin analogue, (D-Ala^2-Met^5)-enkephalinamide (5uM), which blocks IPSPs in these cells without reducing their EPSPs, markedly reduced the LHP. The LHP was also more sensitive to micromolar concentrations of the Ca^{++} antagonist, cadmium, than the EPSP. These observations indicate that the slow-IPSP transmitter may be released from interneurons rather than directly from the afferents. Since antidromic stimulation evokes little LHP, the LHP-transmitter appears to be released from a class of feedforward inhibitory interneurons.

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115.4 THREE TYPES OF HYPERPOLARIZATIONS IN HIPPOCAMPAL CA3 NEURONS *IN VITRO*. W. D. Knowles, P. A. Schwartzkroin and J. H. Schneiderman* Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

The ionic mechanisms of several types of inhibitory hyperpolarizations were studied using intracellular recordings from CA3 pyramidal cells in the guinea pig hippocampal slice preparation (Schwartzkroin, 1975). We studied hyperpolarizations following three different kinds of neuronal activation: 1) stimulation of the afferent mossy fibers; 2) depolarizing intracellular current injection through the recording electrode; and 3) penicillin induced epileptiform bursting. The ionic bases of these hyperpolarizations were investigated in low chloride (22 mM), high potassium (10 mM), 0.2 mM barium, or 10 µM picrotoxin containing media. The results indicated that at least three different inhibitory mechanisms can affect CA3 pyramidal cells. First, subthreshold afferent stimuli produced an early (peak about 17 ms following stimulus) Cl^- dependent, picrotoxin sensitive IPSP with a reversal potential 5-10 mV hyperpolarizing from resting potential. We interpret this to be the IPSP mediated by interneuron synapses near the pyramidal cell soma. Second, in some cells, subthreshold afferent stimulation also elicited a later (peak about 140 ms), long-lasting (200-300 ms) IPSP which was also Cl^- dependent and picrotoxin sensitive. However, this later IPSP was much less voltage sensitive than the early IPSP and was very difficult to reverse. The conductance increase measured at the soma during the late IPSP was smaller than during the early IPSP. We interpret this late IPSP to be generated by inhibitory synapses located on dendrites. The third inhibitory mechanism observed was a long duration (500-1000 ms) hyperpolarization following intracellular current induced bursts. This hyperpolarization was K^+ dependent, Cl^- insensitive and was abolished by Ba^{++}. We interpret this to be a Ca^{++} dependent K^+ current caused by Ca^{++} entry during the burst.

Thus, at least three different hyperpolarizations can occur in CA3 pyramidal cells: early IPSP, late IPSP and Ca^{++} dependent K^+ current. These hyperpolarizations can be separated, but are usually seen in conjunction with each other. For example, suprathreshold afferent stimuli often also activate a Cl^- insensitive hyperpolarization (Ca^{++} dependent K^+ current). Likewise, a portion of the hyperpolarization following a penicillin-induced burst is Cl^- sensitive (early IPSP), while a second portion of the hyperpolarization is K^+ sensitive and Cl^- insensitive.

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115.5 ELECTROPHYSIOLOGICAL PROPERTIES OF GUINEA PIG THALAMIC NEURONS STUDIED IN VITRO. H. Jahnsen and R. Llinas. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York 10016.

In order to study the electrophysiology of neurons in the thalamus 400 µm thick sections were cut from the diencephalon of adult albino guinea pigs and maintained in vitro as previously described (Llinas & Sugimori, J. Physiol. 305:171, 1980). Intracellular recordings were obtained from cells located in both "specific" and "non-specific" nuclei, and their electroresponsiveness was investigated by blocking membrane conductances with tetrodotoxin, tetraethylammonium, 4-aminopyridine, manganese, cobalt and cadmium, and by substitution of ions normally present in the medium with impermeable ions. The recordings revealed that thalamic neurons have two different functional states. At membrane potentials negative to -65 mV, the cells exhibited a burst type of firing pattern when stimulated either directly or via synaptic inputs. The underlying mechanism is a calcium conductance presumably located in the somatic membrane and quite similar to that first described in inferior olive cells (Llinas & Yarom, J. Physiol. 315:569, 1981). At membrane potentials more positive than -60 mV, the cells fired fast spikes repetitively throughout the stimulation much in the same way as motoneurons. When intracellular recordings of presumed dendritic origin were obtained, a second type of calcium electroresponsiveness was seen. As reported in the inferior olive, it had a much higher threshold than the somatic calcium spike. Staining with HRP and Lucifer yellow dye showed that the neurons were either multipolar or had a bipolar appearance. At least some of the cells were "thalamocortical relay cells" since the stained axons could be seen leaving the thalamus toward the cortex. While the sample size studied consisted of more than 300 intracellularly recorded cells, the electrical behavior described above was common to all neurons in all nuclei in the thalamus. This indicates that, as far as the electrical properties are concerned, thalamic neurons must be regarded as electrophysiologically uniform. In conclusion, the electroresponsive properties of the thalamic cell, in particular the ability to switch between two different integrated states with variations of the D.C. membrane potential, probably plays an important role in thalamic function such as that responsible for the recruiting response and for the α and β rhythm in the cerebral cortex. Supported by USPHS grant NS13742 from NINCDS.

115.6 MEMBRANE CURRENTS IN CAT NEOCORTICAL NEURONS IN VITRO. C.E. Stafstrom, P.C. Schwindt*, W.E. Crill, & J.A. Flatman*, Depts. of Physiol. & Biophys., and Medicine, Univ. Wash. Sch. Med. and VA Med. Ctr., Seattle, WA 98195.

Large layer V neurons from area 4γ were studied in in vitro slices of cat neocortex using a single electrode voltage clamp and constant current stimulation. The neurons exhibit a persistent subthreshold Na^+ current which is responsible for inward rectification in the depolarizing direction and which produces a negative slope region on the subthreshold current-voltage curve (Stafstrom et al., Brain Res., 236:221, 1982).

In the presence of TTX, downsteps from depolarizing voltage steps reveal slow, outward current tails that can be blocked by TEA but not Co^{++}. This outward current is activated at potentials near rest and presumably is a slow, voltage-dependent, potassium current. TEA also caused widening of spikes with prolonged depolarizing afterpotentials. Besides blocking synaptic transmission, Co^{++} exposure enhanced cell excitability: bursting behavior was seen in response to depolarizing current pulses, suggesting that a K^+ conductance was being reduced. Co^{++} produces far less spike widening than TEA. After perfusion with both TEA and Co^{++}, cells exhibit large plateau depolarizations that outlast the duration of a stimulus pulse. The plateaus are sustained by the persistent inward Na^+ current which becomes dominant when K^+ conductances are sufficiently depressed, and the plateaus are eliminated by TTX.

In the presence of TTX and TEA, depolarizing current pulses elicit rhythmic action potentials which are blocked by Co^{++} or Cd^{++}, and presumably represent Ca^{++} spikes. The voltage threshold for the Ca^{++} spikes is significantly higher than that for the persistent Na^+ current or for Na^+ spikes. The Ca^{++} influx responsible for the Ca^{++} spikes appears to activate little K^+ conductance, as judged by the shallow afterhyperpolarizations which follow Ca^{++} spikes. The relatively minor role of Ca-dependent K^+ current in these cells is additionally suggested by the Co^{++}-insensitivity of Na^+ spike afterhyperpolarizations and the inability of Co^{++} to eliminate the slow K^+ current tails.

The cells also show pronounced inward rectification upon hyperpolarization. Hyperpolarizing voltage clamp steps reveal a slow inward current underlying this rectification, which we have been unable to block pharmacologically. Thus, time- and voltage-dependent currents are activated by small polarizations on either side of resting potential.

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115.7 4-AMINOPYRIDINE: EFFECTS ON SYNAPTIC TRANSMISSION AND POSTSYNAPTIC CALCIUM CONDUCTANCE IN NEOCORTICAL NEURONS IN VITRO. W.E. Crill, C.E. Stafstrom, & P.C. Schwindt.* Depts. of Physiol. & Biophys., and Medicine, Univ. Wash. Sch. Med. and VA Med. Ctr., Seattle, WA 98195.

Application of 0.5-2.5mM 4-aminopyridine (4AP) to the cat neocortical in vitro slice preparation results in the appearance of giant "unitary" EPSPs in large layer V neurons. These EPSPs have simple waveforms but amplitudes of 5-20 mV; they rapidly increase in frequency, summate, and drive the cells into seizure activity. Such synaptic events are unique to 4AP; they are not seen during Ba^{++} or penicillin-induced seizures, nor after application of TEA, which does not cause seizures in this preparation. The 4AP-induced spontaneous EPSPs disappear and the cells become quiescent after TTX application, indicating that the abnormal transmitter release requires presynaptic action potentials. After synaptic transmission has been abolished by Co^{++}, the addition of 2mM 4AP results in the restoration of evoked EPSPs and the reappearance of the giant spontaneous synaptic potentials.

In contrast to its dramatic presynaptic action, the postsynaptic effect of 4AP is quite mild. In particular, it is difficult to demonstrate a reduction of potassium conductance at these 4AP concentrations in contrast to the clear K^+ conductance depression caused by TEA. TEA (2-10mM) greatly prolongs the spike, eliminates slow outward current tails under voltage clamp, and releases Ca^{++} spikes once Na^+ spikes have been blocked by TTX. 4AP at concentrations of 0.5-2.5mM produces none of these effects. Although higher concentrations of 4AP (12mM) do appear to decrease some K^+ conductance, this reduction is far less than that produced by 10mM TEA. Although 2mM 4AP alone or in addition to TTX cannot release Ca^{++} spikes, the drug does gradually lower the Ca^{++} spike voltage threshold when such spikes are elicited in TTX and TEA. This reduction of Ca^{++} spike threshold appears to be the major postsynaptic effect of 4AP at this concentration. Restoration of Ca^{++} spikes after their blockade by Co^{++} has not been observed in 2mM 4AP in contrast to the ability of this dose to restore Co^{++}-blocked EPSPs.

The intense presynaptic effects of 4AP, as evidenced by abnormal synaptic transmission, contrast markedly with its weak postsynaptic actions. These results suggest that responses to 4AP are not uniform on all neocortical neuronal membranes; rather, certain presynaptic elements (possibly synaptic terminals) are particularly sensitive to the drug. As far as can be judged by postsynaptic effects, the presynaptic action of 4AP at concentrations of 2mM or less is directed toward lowering calcium conductance threshold rather than depressing potassium conductance.

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115.8 ELECTROPHYSIOLOGY OF SINGLE DISSOCIATED CORTICAL NEURONES. Randal Numann*, Robert K.S. Wong, and Robert Clark*. (SPON: B.N. Christensen). Dept. of Physiology and Biophysics, Univ. of Texas Medical Branch, Galveston, TX 77550.

Single enzymatically dispersed neurones have been obtained from frog and mammalian cortices. Tissue cubes ($2mm^3$) were prepared from frog paleocortex and guinea pig hippocampus. They were treated for 1 hour with 5% papain in solutions of similar ionic composition to those used in electrophysiological studies of frog and mammalian CNS. Cells were then dispersed mechanically using a fire-polished pasteur pipette. Single neurones thus obtained had discrete somata and retained their proximal processes. Pyramidal cells could be identified by their characteristic triangular cell body (20-40 µm diam.) and several apical dendrites (up to 10 µm diam.).

Intracellular recordings were obtained using low resistance electrodes (15-20 MΩ) which were first positioned to touch the cell surface. Negative pressure was then applied to the inside of the electrode to rupture the cell membrane, forming a high resistance seal. Single channel currents were also recorded from these cells using the patch clamp technique described by Neher and Sakmann.

Spontaneous activity has been recorded from both frog and guinea pig neurones. The frog cells had resting potentials of about 60 mV and fired in single action potentials and bursts of 5-6 action potentials (65 mV amplitude) superimposed on a depolarising envelope. Hippocampal neurones (of both pyramidal and granule cell types) had resting potentials of about 50 mV and exhibited spontaneous overshooting action potentials.

After hyperpolarizations lasting more than 500 msec followed the burst recorded in frog neurones suggesting that these cells might possess a Ca^{++} dependent K^+ conductance. Initial patch clamp studies support this hypothesis. Single channel currents were activated by membrane depolarization. Their amplitude was voltage dependent and they had a conductance of 103 pS. The reversal potential was estimated to be -86 mV. Channel open time increased exponentially with increasing depolarizing voltage. These single channel current properties resemble those for Ca^{++} induced K^+ channels described elsewhere.

This data suggests that a detailed analysis of the membrane function of mammalian cortical neurones is now possible. We anticipate that such studies addressed to neuronal activity and transmitter action will contribute to the understanding of the process of integration in the CNS.

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115.9 ENHANCED EXCITABILITY FOLLOWING AXOTOMY OF A CENTRAL VERTEBRATE NEURON. M. Titmus* and D.S. Faber (SPON: P.G. Funch). Div. Neurobiology; Dept. Physiology; SUNYAB; Buffalo, NY 14214.

A number of neurons, including spinal motoneurons and some identified invertebrate cells, exhibit a transiently increased electrogenecity of normally passive soma or dendritic membranes following axotomy. The goldfish Mauthner (M-) cell, an identifiable medullary neuron, evokes a similar but persistent increase in soma-dendritic (SD) excitability following axotomy by spinal transection 8-10 mm caudal to the cell body (Faber and Zottoli, Brain Res., 223:436-443, 1981). In this case spike height recorded in the soma is often increased by 40 to 150% at 40 to 200 days. We now further characterize this induced excitability, including its ionic basis. Simultaneous intracellular recordings were obtained from the M-cell soma and lateral dendrite of impulses elicited either antidromically or directly by current injection through one microelectrode. Normally, M-cell spikes recorded in the soma are generated in the initial segment-axon hillock (IS-AH) region. Increased excitability was detected by either a spike height greater than 50 mV and/or the presence of additional components due to delayed activation of the soma-dendritic membrane. The SD component often exhibited spontaneous amplitude fluctuations which could be as great as 10 mV. Electronically differentiated representations of these impulses generally revealed two peaks on the rising phase, corresponding to the IS-AH spike and the added SD component. The maximum rate of rise of the IS-AH spike (140 V/sec) was comparable to that of control cells. The SD spike, when clearly distinguished, was at least equally fast. The amplitude fluctuations in the SD spike are not due to contributions of discrete patches of excitable membrane localized to the distal lateral dendrite, as the component amplitudes are maximal at proximal recording sites. Rather, the smaller inferior and cap dendrites arising from the soma may be involved.

Voltage sensitive sodium, calcium and potassium conductances contribute to the IS-AH spike of control M-cells. When the brain was superfused with saline containing TTX ($1x10^{-6}$M) both the IS-AH and SD spike components of axotomized M-cells were abolished, with the latter being blocked first. In contrast, Co^{++} (25-45 mM) or Mn^{++} (20 mM) produced only slight decreases in component amplitudes. Finally, as with control M-cells, intracellular injection of a TEA, 4-AP mixture caused a cobalt-sensitive increase in spike duration. These results suggest that the primary effect of axotomy on spike electrogenesis is due to the appearance of voltage sensitive sodium channels in the somatic membranes. It is not yet clear whether there are comparable alterations in the distribution of calcium and potassium channels.

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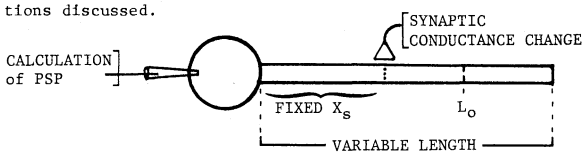
115.10 EFFECT OF DENDRITIC LENGTH UPON SYNAPTIC EFFICACY. John P. Miller and Wilfrid Rall. Zoology Dept., Univ. of Calif., Berkeley, CA. 94720, and Math Research Branch, NIADDK, NIH, Bethesda, MD. 20205.

The dendrites of some types of neurons seem to be "unreasonably" long, considering their functional roles in synaptic integration. For example, the average electrotonic length of cat spinal motoneuron dendrites is about 1.5 length constants. This means that steady state voltages are attenuated by 78% along the length of such a dendrite. The amplitudes of transient potentials are attenuated to a much greater extent, due to the capacitive filtering by the dendritic membrane. So why are such dendrites this long, if synaptic input at their tips contribute so little to integrative processes at the spike initiation zone? In previous studies, investigators have usually approached this general question by calculating the dependance of synaptic efficacy upon synaptic location. Using mathematical models of simple neurons, this is accomplished by varying the location of synapses along dendrites of fixed length. We have taken an alternate approach, and have calculated the dependance of synaptic efficacy upon total dendritic length. Using a compartmental computer model of a simple neuron, we 1) fixed the location of a synaptic contact on one dendrite, and 2) varied the length of dendrite extending beyond the point of synaptic contact. We obtained the somewhat surprising result that the extension or "growth" of the dendrite beyond the point of synaptic contact can increase the efficacy of that synapse.

For a dendrite having particular values of Rm, Cm, Ri, and diameter, and having a single synaptic contact at distances "Xs" from the soma, there exists an "optimal" total dendritic length "Lo" for the dendrite, i.e., a length Lo for which the amount of charge reaching the cell body, due to a synaptic conductance change at distances Xs, is maximized. For small amplitude PSP's, the optimal length Lo simply equals Xs, i.e., the optimal dendritic length is the minimal dendritic length. However, for large amplitude synaptic conductance changes (those resulting in local PSP amplitudes that are a substantial fraction of the synaptic driving potential) the optimal length Lo may be substantially greater than Xs. Thus we speculate that some dendrites may be "long" in order to increase the efficacy of proximal synapses. The basis of this effect will be presented, and several implications discussed.



115.11 THEORETICAL MODELS WHICH INCREASE Rm WITH DENDRITIC DISTANCE HELP FIT LOWER VALUE FOR Cm. W. Rall. Math. Research Br., NIADDK, NIH, Bethesda, MD 20205.

Combined anatomical and electrophysiological data for individual dendritic neurons have recently been obtained and analysed by several investigators (1, 2, 3). The estimation of membrane parameter values from such data depends upon the choice of simplifying assumptions incorporated in the theoretical model (1-6). Assuming uniform passive membrane properties, the resulting estimate of Rm for each neuron, together with the estimate of τ_m for the same neuron, has usually implied Cm values that are 2 or 3 times the 1 $\mu F/cm^2$ value established in the 30s by Cole, Curtis and Fricke (7). Discussions with colleagues, Burke, Miller, and Rinzel, led me to explore both compartmental and continuous neuron models in which the value of Rm increases with distance from the soma in each dendritic tree, while the value of Cm is held uniform. Such models have system time constants which can fit the known experimental constraints with Cm values closer to the 1 $\mu F/cm^2$ value of Cole (7).

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115.12 CELL SIZE AND SPECIFIC MEMBRANE RESISITIVITY OF TYPE-IDENTIFIED CAT SPINAL MOTONEURONS: MORPHOLOGICAL, PHYSIOLOGICAL AND MODELING STUDIES. J.W. Fleshman, R.E. Burke, L.L. Glenn, A. Lev-Tov* and John P. Miller. Lab. of Neural Control, NINCDS, and Math. Research Branch, NIADDK, NIH, Bethesda, MD 20205.

Intracellular injection of horseradish peroxidase (HRP) permits correlative studies of the morphological and electrophysiological properties of functionally-identified neurons. Initially, we measured the dimensions of somas and stem dendrites of 57 ankle extensor α-motoneurons which were identified as to motor unit type. The total surface area (A_N) of each cell was estimated from the average soma diameter, plus the numbers and diameters of stem dendrites, using the recently reported linear correlation between dendritic membrane area and stem diameter (Ulfhake and Kellerth, J. Comp. Neurol. 202:571, 1981). Average soma diameters did not differ significantly between FF, FR and S motoneuron groups. However, A_N did exhibit significant differences between unit types, decreasing in the order FF>FR>S (mean A_N: 369, 323 and 250 x 10^3 μm^2, respectively). Motoneuron input resistance (R_N) was also measured in a subset of 19 cells (8 FF, 2 FR, 9 S). Assuming that α-motoneurons can be represented by an equivalent membrane cylinder with a lumped electrotonic length (L) that is invariant with motor unit type (see Burke and ten Bruggencate, J. Physiol. 212:1, 1971), the relation between estimated A_N and measured R_N implied that the specific membrane resistivity (R_m) of type S motoneurons is 2 - 3 times greater than that of type F cells. In order to examine this possibility further, we are currently reconstructing and measuring the somas and dendritic trees of HRP-filled, type-identified motoneurons in which electrophysiological estimates of R_N, membrane time constant, and L were obtained. The morphological data is used to construct a computer-based compartmental model of the individual neurons, in which various choices of values for R_m and specific membrane capacitance (C_m) can be tested. The results of such tests can then be matched against the electrophysiological data from the same neurons. In principle, this approach should constrain the possible choices of R_m and C_m in α-motoneurons and permit testing the hypothesis that R_m may vary from one region to another within a given neuron, as well as between different classes of motoneurons.

116.1 TYPE I, ASYMETRIC SYNAPSES FROM THE RAT CEREBELLUM. D. E. Groswald, P. R. Montgomery and P. T. Kelly. Div. of Biol., Kansas State Univ., Manhattan, KS 66506.

Synaptic junctional(SJ) and synaptic plasma membrane(SPM) fractions isolated from rat forebrain(FB) and cerebellum(CBLM) have been compared for subcellular purity based on morphology of synaptic structures, and for their protein and glycoprotein(GP) composition. SJ fractions from FB and CBLM were of comparable purity. The main morphological difference between CBLM and FB SJs is that CBLM postsynaptic densities(PSD) are consistently thinner when compared to FB PSDs.

SDS-gel ananlysis showed that SJ fractions from CBLM and FB contained equivalent numbers of proteins that possessed similar molecular weights and staining intensities. Both SPM and SJ fractions from FB and CBLM contain tubulin and actin; proteins which contribute in part to the synaptic junctional cytoskeleton, but some prominent differences were apparent. Most notably is a 235K glycoprotein(GP-235) that is a major component of CBLM SJs and is not detectable in FB. CBLM SJs contained proportionately less tubulin and the 52K major PSD protein(mPSDp) when compared to FB SJs.

Using the increased resolution of two-dimensional PAGE, the protein composition of CBLM and FB SJ fractions are very similar. Similarities in polypeptide composition between SJ fractions from CBLM and FB were examined by peptide mapping of individual synaptic junctional proteins. The 52K protein in CBLM SJs, which is 10-15 times less when compared to the FB SJ, was found to have a peptide composition similar to that of the mPSDp.

Mannose containing GPs were examined by the binding of Concanavalin A(Con A) to SDS-gels. A significant enrichment occurs in both FB and CBLM SJ fractions of GPs that reside in the postsynaptic membrane. Without exception, the prominent GPs characteristic of FB SJ fractions are either absent or present in greatly reduced amounts in SJ fractions isolated from CBLM. In contrast to FB, CBLM SJs contain their own distinct group of Con A binding GPs with M_rs of 235K, 195K, 190K, 125K, 115K, 105K and 93K. When analyzed by peptide fingerprinting, the CBLM-specific, GP-235K polypeptide is highly related to a fodrin-like protein doublet(Levine and Willard, JCB, Vol. 90, 1981), which is present in both FB and CBLM SJ fractions.

The greatly reduced quantities of mPSDp in CBLM SJs and the marked differences in Con A binding GPs between FB and CBLM SJ fractions indicates that CBLM does not contain significant amounts of FB asymmetric, type I synaptic junctions. These results show that the cerebellum contains a new class of type I, asymmetric synaptic junctions.

116.2 BINDING OF D-[^{3}H]GLUCOSE TO RAT SYNAPTIC MEMBRANES. Jayne T. Kyle and Barry I. Gold. Dept. of Pharmacol. Uniformed Services Univ., Sch. of Med., Bethesda, MD 20814.

We have been studying glucose transport by brain using the uptake of [^{3}H]2-deoxyglucose ([^{3}H]2DG) by slices of rat cerebral cortex as an in vitro model. Our finding that net uptake of [^{3}H]2DG was inhibited in the presence of 1.0 mM DNP led us to hypothesize that glucose transport by brain may be dependent, in part, on mitochondrial energy. To augment our work in brain slices, we have begun to study the association of D-[^{3}H]glucose with a synaptic membrane fraction prepared from rat brain.

Rat cerebral cortex was freshly dissected, and homogenized in ice-cold 0.32 M sucrose. A synaptic membrane-enriched fraction (lysed P_2) was prepared similar to the method of Enna and Snyder (Brain Res. 100:81,1975). Membranes were suspended in 25 mM Hepes buffer, pH 7.4, and pipetted into plastic tubes. Hepes or Hepes containing D-glucose was added to a final glucose concentration of 50μM and the samples were equilibrated at 37°. Binding was initiated by adding D-[^{3}H]glucose and samples were incubated at 37°. After 15 minutes, the samples were cooled and immediately centrifuged; the resulting pellet was rinsed, solubilized in Protosol, and radioactivity was estimated by liquid scintillation spectrometry. In some studies, binding was terminated by rapid filtration over GF/B filters; the filters were rinsed, tissue was solubilized in Protosol, and radioactivity was estimated.

D-[^{3}H]glucose binding to synaptic membranes was stereospecific; binding was maximally displaced in the presence of 50 μM D-glucose but was not displaced by equimolar L-glucose. 2-Deoxyglucose and 3-O-methylglucose inhibited D-[^{3}H]glucose binding with IC50's of 1.3 μM and 13.0 μM respectively. Saturation kinetics showed an apparent K_d for D-[^{3}H]Glucose of 0.3 μM, estimated from the Scatchard plot. At 37°, specific D-[^{3}H]glucose binding appeared to reach equilibrium at 10 min. From initial studies of association and dissociation rate constants, we graphically estimated K_{+1}=0.023 $nM^{-1}min^{-1}$ and K_{-1}=0.046 min^{-1}. Specific D-[^{3}H]glucose binding was not affected in the presence of 100 μM phlorizin or 100 μM DNP. Binding was inhibited by 50% in the presence of 1.0 mM ADP, and binding was inhibited by 80% in the presence of 1.0 mM ATP. In a preliminary study of the subcellular localization of the binding site, we observed stereospecific D-[^{3}H]glucose binding to highly purified synaptic membrane-enriched fractions, and to membrane fractions derived from purified mitochondria. While these results begin to characterize a recognition site for glucose in brain, the role of this recognition site remains to be determined.

116.3 IMMUNOCYTOCHEMICAL LOCALIZATION OF SODIUM-POTASSIUM ADENOSINE TRIPHOSPHATASE IN THE RAT CENTRAL NERVOUS SYSTEM. R.G. Ariyasu*, M.H. Ellisman, J.A. Nichol*, and T.J. Deerinck*. (SPON: C.E. Spooner). Dept. of Neurosciences, School of Medicine, Univ. of Calif., San Diego, La Jolla, CA 92093.

Evidence that the concentration of intracellular sodium ions influences many cellular processes has increased interest in localizing sodium-potassium adenosine triphosphatase [(Na^++K^+) ATPase]. Recent reports have immunocytochemically localized this enzyme in goldfish optic nerve (Schwartz, M., et. al., J. Neurochem., 36: 107, 1981) and in knifefish CNS (Wood, J.G., et. al., J. Neurocytol., 6: 571, 1977). Unfortunately antibodies raised against (Na^++K^+) ATPase from canine kidney (Kyte, J., J. Cell Biol., 68: 287, 1976), or Electrophorus electroplax do not cross-react significantly with forms of the enzyme found in rat. Biochemical and immunocytochemical advances have enabled us to raise antibodies specific for rat (Na^++K^+) ATPase and utilize these to localize the enzyme in the rat CNS.

Purification of the antigen from rat renal medulla involved preparation of microsomes (Kyte, J., J. Biol. Chem., 246: 4157, 1971; Winslow, J.W., J. Biol. Chem., 256: 9522, 1981) which were solubilized with sodium dodecyl sulphate (SDS) (Jorgensen, P.L., Biochem. Biophys. Acta., 356, 36, 1974) at an optimal concentration determined for each preparation. (Na^++K^+) ATPase was purified from the suspension by sucrose gradient centrifugation. One hundred micrograms of this material were injected into lymph nodes of rabbits, and sera collected after four to six weeks. These sera were precipitated with ammonium sulphate and the resuspended pellet passed through a DEAE-cellulose column to partially purify the IgG fraction. Booster inoculations of 20-50mg were given at 1-3 month intervals until sera demonstrated high titers in enzyme linked immunosorbant assays (ELISA) using the purified antigen. High specificity of antibodies for antigen was demonstrated by immunoassays using nitrocellulose paper blots of polyacrylamide gels (Western blots).

These antibodies have been employed with the peroxidase-antiperoxidase technique to localize the enzyme to the basolateral and luminal surfaces of the proximal and distal epithelial cells of the rat kidney. They also cross react with glial and neuronal forms of the antigen in rat CNS. Neuronal soma, nodes of Ranvier and astrocytic processes demonstrate the presence of (Na^++K^+) ATPase. In addition, these antibodies cross-react with mouse renal and neuronal forms of the (Na^++K^+) ATPase, enabling investigations into the distribution of (Na^++K^+) ATPase in genetically pathologic mice and rats. Supported by NIH grant PHSGM07198 to RGA and NIH NS14718, MDA and NMSS grants to MHE.

116.4 CELLULAR LOCALIZATION OF ACETYLCHOLINESTERASE IN CULTURED EMBRYONIC RAT MYOTUBES S. K. Brockman*, R. J. Przybylski, and S. G. Younkin, Depts. of Pharmacology and Anatomy, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106

Experiments were performed on 8-day myotube cultures derived from 20-day rat embryos. Three methods were used to measure the relative amounts of intracellular and external AChE in myotubes at 2° C: 1) 1.0 μM echothiophate, a cationic phosphorylating agent, was added to inactivate external AChE selectively, 2) 10 μM methanesulfonyl fluoride (MSF) and 100 μM decamethonium were added to inactivate external enzyme selectively, and 3) the rate of hydrolysis of ^{3}H-ACh added to the medium bathing intact myotubes was measured to evaluate the activity of external enzyme directly. The results from these methods are in excellent agreement and indicate that 26% of the AChE is external and 74% is intracellular. To isolate intracellular AChE, external AChE was irreversibly inactivated by treating myotubes at 2° C with 10 μM MSF and 100 μM decamethonium for one hour. To isolate external AChE, myotubes at 2° C were first exposed to 1.0 μM echothiophate for 15 min to protect (diethylphosphorylate) external AChE and then treated with 1.0 mM methanesulfonyl fluoride for 30 min at 37° C to irreversibly inactivate intracellular AChE. Control myotubes and myotubes in which the external or intracellular enzyme had been isolated were sequentially extracted to separate globular, asymmetric, and non-extractable AChE. Individual globular and asymmetric forms were then analyzed by velocity sedimentation on sucrose gradients. The fractions in which external enzyme had been isolated as diethylphosphorylated AChE were reactivated with 2-PAM prior to analysis. Our data indicate that intracellular and whole myotube AChE have similar compositions. Intracellular AChE is 79% globular forms (20% 10S, 59% 4S), 17% asymmetric forms (6% 16S, 5% 12.5S, 7% other), and 4% non-extractable enzyme. Whole myotube AChE is 75% globular forms (21% 10S, 54% 4S), 18% asymmetric forms (6% 16S, 6% 12.5S, and 7% other), and 6% non-extractable enzyme. Intracellular and external AChE have different compositions. External AChE is 55% globular forms (26% 10S, 29% 4S), 32% asymmetric forms (12% 16S, 10% 12.5S, 10% other), and 13% non-extractable enzyme, so external enzyme is composed of relatively more 10S, 16S, 12.5S, and non-extractable AChE, and relatively less 4S AChE. The most striking finding to emerge from this study is that the globular and asymmetric forms are all predominantly intracellular in cultured embryonic rat myotubes. Our results indicate that 85% of the 4S, 69% of the 10S, 59% of the 16S, and 56% of the 12.5S forms are intracellular. These results support the hypothesis that the 10S and asymmetric forms of AChE are assembled intracellularly.

116.5 ONTOGENETIC CHANGES IN ACETYLCHOLINESTERASE AND IN ITS TRANSLATABLE mRNA IN NORMAL AND IRRADIATION-AGRANULATED RAT CEREBELLUM. H. Soreq, R. Parvari* and I. Silman*. Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel.

Major changes in levels and histochemical localization of acetylcholinesterase (AChE) occur in developing rat cerebellum (*cer*). Assignment of cholinergic and cholinoceptive functions to specific cell types during development is still a matter of controversy. As a novel approach to this problem, we have determined, in normal and in irradiation-agranulated developing *cer*, AChE levels, which should include contributions of incoming fibers, and levels of mRNA species directing AChE synthesis (AChEmRNA), which should reflect the biosynthetic potential of endogenous cell bodies. Cholinesterase (ChE) activity increases 3-fold from day 1 to day 38, to reach ca. 3 nmole/min/mg tissue ^{3}H-ACh degraded. Irradiation-induced agranulation results in consistently higher specific ChE activity, reaching 4.5 nmol/min/mg at day 38. However, total ChE in the agranulated and normal *cer* is equal. At day 1, 30% of ChE is pseudocholinesterase (ψChE), and 70% AChE. By day 10, ψChE decreases to 15% of total ChE, remaining at 10-15% thereafter. On sucrose gradients of Triton-NaCl extracts, ψChE migrates as a 4S peak, whereas the main AChE species is 9S throughout development in both normal and irradiated *cer*. Although AChE activity in mature *cer* is known to reside primarily in the granular layer, agranulation does not alter content, substrate specificity or sedimentation characteristics of AChE, nor does it affect ontogenetic changes in these parameters. Levels of AChEmRNA were determined by a *Xenopus* oocyte microinjection assay and a radiometric ChE assay. Throughout ontogenesis, newly synthesized ChE, directed by cerebellar mRNA, was primarily AChE. Maximal AChEmRNA levels were detected at day 1, when 50 ng poly(A)$^+$RNA per oocyte direct, during 24 hr oocyte incubation, production of AChE capable of degrading ca. 15 pmol/min ACh. In 10 and 60 day *cer*, corresponding values decrease to ca. 5 pmol/oocyte/min, and a value of ca. 2 pmol/oocyte/min is reached for 90 day *cer* mRNA. The ontogenetic decrease in AChEmRNA may be ascribed to the known disappearance of AChE from maturing Purkinje cells, as well as to proliferation of the non-cholinergic granular neurons masking contributions of non-abundant cholinergic neurons (e.g. Golgi type II cells). Throughout maturation, mRNA from control and irradiated *cer* direct production of AChE with similar efficiency. Irradiation-induced agranulation, in contrast to the known effects of physical deafferentation, seems not to alter expression of AChE in the maturing *cer*. Thus, an approach combining biochemical assays with mRNA expression can yield insight into contributions of exogenous and endogenous neuronal populations to maturation of interconnections in developing brain regions. (Supported by the MDA.)

116.6 MONOCLONAL ANTIBODIES AGAINST SYNAPTOSOMAL PLASMA MEMBRANES OF RAT HIPPOCAMPUS. R.Hofstein and C.J.Barnstable (SPON: P. MacLeish). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

As part of a continuing effort to identify markers of cell-types and synaptic junctions in the CNS we have made antibodies to synaptosomal plasma membranes of rat hippocampus (HPC-SPM). This region has the advantages that it consists of well defined cell types, namely pyramidal and granular cells, and can be dissected out with very little contribution of extrahippocampal tissue. Purified HPC-SPM were prepared and used to immunise Balb/C mice. Spleen cells of these mice were fused with P3 X63-Ag8.653 myeloma cells and hybrid cultures selected in HAT medium.

In one fusion, over 400 cultures were screened by indirect solid phase binding assay using radioiodinated F(ab)$_2$ fraction of goat anti-mouse immunoglobulins. More than 50% of the tested cell-culture supernatants were found to be positive when tested against HPC-SPM and these were further screened by immunofluorescence using 10um sections of rat brain. Most of the antibodies reacted in a nonselective manner but five were very selective in their distribution. These were further characterized. All five, namely HPC-1,HPC-2,HPC-7,HPC-10 and HPC-12, were cloned twice by limiting dilution. These antibodies were retested for neural specificity by quantitative inhibition assays using HPC-SPM and liver membranes. In these assays there was no inhibition by liver membranes. The patterns of reactivity within certain areas of the central nervous system were studied by indirect immunofluorescence. HPC-1 stained mainly the molecular layer of CA1 and CA2 of the hippocampus . HPC-2 and HPC-12 stained intensely the molecular layer and around the somata in hippocampus as well as area dentata. All of these three antibodies appeared to be nonreactive with cells in the striatum, cerebellum and the retina. HPC-10 binds to several cell types. The antigen seems to be concentrated on apical dendrites of pyramidal cells of hippocampus,on granule cells of dentate, retinal photoreceptors and granule cells of cerebellum. Finally, HPC-7 has a wide range of distribution and stains mainly fibers in all sections including incoming fibers of cerebellum.

Our approach of using characterised subcellular fractions of well defined brain regions such as the hippocampus has given rise to antibodies which are a powerful tool in identifying and localising markers of particular cell types. Some of these are confined to distinct structures while others can be found on particular cell types in various brain regions. Experiments are in progress to charactize the biochemical properties of the various antigens recognized by our antibodies. (Supported by Chaim Weizmann fellowship to RH and grants NS17309 and EY03705 to CB).

116.7 SOME MONOCLONAL ANTIBODIES WHICH RECOGNIZE LEECH NEURONS ALSO DISTINGUISH CLASSES OF PERIPHERAL EPITHELIUM. B. Zipser, N. Hogg*, J. Smart*, F. Hendrickson*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

The antigens recognized by three mAbs of interest because of their CNS reactivities were further characterized for reactivity against gut and penis tissue and also biochemically using Western blots and lectin column binding. Antibody Lan3-8 (Zipser, McKay, Nature, 1981) recognizes an internal antigen while Lan3-2 (Zipser, McKay) and Laz2-369 (Hogg, Zipser, in prep.) react with antigens found on the surface. In the CNS, mAb Lan3-8 reacts with all neurons, Lan3-2 reacts strongly with 4 nociceptive neurons while Laz2-369 reacts strongly with only two of these nociceptive neurons. In the gut, Lan3-8 reacts with some neurons but not with epithelium. Lan3-2 weakly labels neurons and strongly labels epithelial patches. Laz2-369 strongly labels neurons and epithelial patches. In the penis, Lan3-8 labels only epithelial cells while the other two do not react at all.

The molecular weights of the antigens from various tissues were compared on Western blots. Lan3-8 recognizes the same single high molecular weight antigen in extracts from either CNS, gut and penis. The other two mAbs react with multiple antigen bands. The CNS pattern of antigen bands recognized by Lan3-2 and Laz2-369 have some bands in common. The gut patterns also have bands in common. However, there appear to be no common bands when the CNS is compared with the gut using these two antibodies.

The surface antigens recognized by Lan3-2 in CNS extracts were further characterized by lectin binding experiments using sequential affinity chromatography with Lens culinaris lectin (specific for D-mannose) and Ricinus communis (specific for terminal D-galactose) (Smart, Hendrickson, Zipser, in prep.) This revealed that at least two of the three antigens with apparent molecular weights between 200,000 and 130,000 daltons had mannose containing side chains. They do not appear to have galactose either as the terminal or penultimate residue.

116.8 RAPID BA^{2+}-INDUCED CHANGES IN CONTRAST AT THE SURFACES OF RAT ADRENAL CHROMAFFIN CELLS OBSERVED WITH VIDEO-ENHANCED NOMARSKI MICROSCOPY. C. Edwards, H. Ye* and D. Englert*. Dept. of Biol. Sci., SUNY Albany, 1400 Washington Ave., Albany, NY 12222.

High resolution video-enhanced Nomarski differential interference contrast microscopy has been used to observe phenomena possibly related to exocytotic secretion from rat adrenal chromaffin cells. A plane of focus at the surface of the cell was observed while the cell was perfused with a Ca^{2+}-free solution containing 3.4 mM Mg^{2+} initially, followed by 3.4 mM Ba^{2+}. Ba^{2+}, but not Mg^{2+}, is known to cause massive exocytotic secretion from adrenal chromaffin cells. The most frequently observed phenomenon in the Ba^{2+} solution was the sudden appearance of a vesicle-like particle of about 0.5 μm or less in diameter; this was rarely observed in the Mg^{2+} solution. The shadow casting of the particles usually appeared to be opposite to that of the edges of the cell, indicating structures with densities less than their surroundings.

Supported by a grant from NIH, NS-07681.

116.9 SKELETAL MUSCLE CELL MYOGENESIS IN VITRO: THE EXPRESSION OF EXTRACELLULAR MATRIX PROTEINS. R. L. Beach, W. V. Burton* J. Hamilton*, and B. W. Festoff. Veterans Administration Medical Center, Kansas City, MO and Departments of Neurology and Biochemistry, University of Kansas Medical Center, Kansas City, KS

Although development, maintenance and regeneration of both skeletal muscle and neuromuscular synapses require important interactions with the extracellular matrix (ECM), the current literature does not allow a clear determination of which cellular elements produce the various components of muscle ECM. To identify the role of muscle cells in this regard we have studied the production of ECM proteins and their incorporation into an insoluble matrix by a clonal skeletal muscle cell line (G8-1). Fibronectin (fn), collagen, actin, myosin and laminin were identified as components of an insoluble, substrate-attached ECM produced by these cells. Electron microscopy of multinucleated myotubes (mt) showed a basal lamina not apparent in myoblasts (mb) or in monoucleate cells in mt cultures. Formation of ECM by these cells was strongly dependent on ascorbic acid. Immunocytochemical studies demonstrated that lamin and type IV collagen were enriched in mt relative to non-fused cells, and were associated with the surface of mt. Myotube cultures secreted about 7 times the peptidyl hydroxyproline (OH) secreted by mb cultures. Gel electrophoretic and immunochemical analyses of media demonstrated that these cells synthesized and secreted fn, lamin and collagens. We have further characterized the collagen species secreted by these and other (CNBr digestion, selective precipitation, and 3- and 4- OH proline analyses) techniques. The major pepsin resistant, collagenase-sensitive protein in media migrated similarily to α1(I), had the major CNBr peptides of α1(I) with levels of 3- and 4- OH proline similar to those previously reported for α1(I) trimer. α1(I) trimer was the predominant collagenous species in the media with additional evidence for the presence of both types III and IV collagens. Collagen was apparent in both cellular and ECM preparations under conditions where collagen fibrils and basal lamina were observed ultrastructurally. 3- to 4-OH proline ratios in mt cultures were higher than in mb cultures and were consistent with mt associated basement membrane collagens. We have also analyzed the types of cell-associated ECM proteins extractable from mb and mt cultures. These studies show the differentiation-dependent expression of basement membrane components of the ECM by clonal skeletal muscle cell.

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116.10 IN VITRO STUDIES OF THE SKELETAL MUSCLE COXSACKIE A_2 VIRUS RECEPTOR, C.G.Andrew, D.B.Drachman, O.Narayan* The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Coxsackie A_2 virus is capable of infecting denervated adult skeletal muscle as well as immature fetal muscle, producing an inflammatory myopathy in vivo. Infection also occurs in cultured embryonic skeletal muscle but only during specific phases of myogenesis. Using a purified Coxsackie A_2 virus radiolabeled in vitro to high specific activity we have examined the ability of primary muscle cultures to bind and take up this virus.

Undifferentiated myogenic cells (presumptive myoblasts and myoblasts) were found to contain surface receptors for this virus. Binding occurred over 3 hours at 4° C and thereafter leveled off. Uptake of the virus by cultures at 37° C occurred at three times the rate of binding, presumably reflecting endocytosis. Cultures prebound with virus at 4° and then shifted to 37° demonstrated an immediate increase in viral uptake within 15 minutes. Radiolabeled virus binding could be inhibited by addition of purified unlabeled virus. Anti-Coxsackie A_2 virus antibody effectively eliminated binding.

We have conducted a preliminary characterization of the chemical nature of the in situ Coxsackievirus receptor. Because of evidence suggesting that other viral receptors are plasma membrane glycoproteins, we began by investigating the effect of a variety of lectins and glycosidases on subsequent binding of radiolabeled virus to cultured muscle cells. Of eight lectins and six glycosidases examined, wheat germ agglutinin and N-acetylglucosaminidase were found to effectively inhibit Coxsackie A_2 virus binding. No effect was produced by either agent on the ability of the cultures to bind α-bungarotoxin.

Thus, the Coxsackie A_2 virus receptor would appear to be a constituent sarcolemmal glycoprotein containing a terminal N-acetylglucosamine. Radiolabeled Coxsackie A_2 virus provides a probe for future characterization of a surface receptor under developmental control and present in undifferentiated myogenic cells.

116.11 EFFECTS OF CALCIUM ON PHOSPHORYLATION OF ERYTHROCYTE GHOST MEMBRANES DERIVED FROM ETHANOL-DEPENDENT RATS. M. Virmani*, H.C.Pant and E. Majchrowicz*, (Spon: G. C. Salmoiraghi). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Protein phosphorylation has been proposed to be one of the basic mechanisms controlling cellular function. The effects of calcium at micromolar concentrations on protein degradation and phosphorylation were studied in erythrocyte ghost membranes derived from control and ethanol treated rats. Tolerance and physical dependence upon ethanol were induced in male Sprague-Dawley rats (250-350 g) as previously described (Majchrowicz, E., Psychopharmacologia, 43: 245, 1975). Ethanol (20%, w/v) 8-11 g/kg/day, was administered in 6-9 fractions by oral intubation for 4 days. Four groups of rats were used: (1) controls (water treated); (2) dependent-intoxicated (prodromal detoxication phase); (3) dependent-withdrawing (ethanol withdrawal syndrome); and (4) acute administration (single dose of 6g/kg). Erythrocyte ghost membranes were isolated and phosphorylated with [γ-^{32}P]-ATP. The protein distribution and phosphoprotein patterns were determined by polyacrylamide gel electrophoresis (PAGE) and autoradiography. Coomassie blue staining indicated that the general charactristics and distribution pattern of the major protein bands (Bands 1, 2, 2.3,* 3, 4.1, 4.2, 5, 6, 7, and 8) were similar in all four experimental groups. However, in the presence of 0.8 mM Ca^{2+} and 1 mM EGTA there was a small decrease in Band 2.3 which was more pronounced at higher Ca^{2+} concentrations in all four experimental groups. This decrease was due to Ca-activated proteolysis of this protein. The phosphorylation of Spectrin (Band 2) in the presence of 0 mM $CaCl_2$ and 1 mM EGTA was 150$\pm$20% higher than controls in dependent-intoxicated rats and 125$\pm$11% higher in dependent-withdrawing rats. After a single acute dose of ethanol there was little change in Spectrin compared with the controls. Pronounced effects were observed after addition of $CaCl_2$ (0.8 mM + 1 mM EGTA) to membrane preparations. The phosphorylation of Spectrin in controls was decreased to 20$\pm$14% of the value in the absence of Ca^{2+}, and in animals receiving a single acute dose of ethanol to 40$\pm$15%. However, in both dependent-intoxicated and withdrawing rats the phosphorylation of Spectrin was only reduced to 70$\pm$15%. These data suggest that the induction of tolerance and physical dependence upon ethanol are associated with the development of resistance to the effects of calcium on the physical and chemical properties of erythrocyte ghost membranes.

116.12 A ROLE FOR STEROL IN OLIGODENDROGLIAL DIFFERENTIATION. J.J. Volpe and K.A. Obert.* Depts. of Ped., Neurol., Biol. Chem., Washington Univ. School of Med., St. Louis, MO 63110

Cholesterol is a major constituent of glial cell membranes. However, the roles of this lipid in these cells remain essentially undefined. In order to evaluate the role of cholesterol in glial differentiation, we developed conditions for inducing oligodendroglial differentiation of cultured C-6 glial cells and, then, utilized a specific inhibitor (ML-236B) of the rate-limiting enzyme in cholesterol biosynthesis (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase) to evaluate the relation of membrane sterol to this differentiation.

C-6 glial cells were induced to undergo morphological and biochemical differentiation by growth in serum-free medium. Under these conditions, within 48 hrs the cells develop long, discrete cytoplasmic processes and a 3-4-fold induction of the oligodendroglial-myelin-specific enzyme, 2',3'-cyclic nucleotide phosphohydrolase (CNPase). We determined whether alteration in sterol content of the cells prior to exposure to serum-free medium prevented the differentiation just described. A concentration-dependent decrease in sterol content and, as a consequence, the sterol/phospholipid molar ratio was accomplished by treating the cells for 24 hrs with ML-236B under conditions of (1) increased sterol demand (cell proliferation) and (2) absence of exogenous cholesterol (growth in 10% lipoprotein-poor serum). Over the 48 hrs following removal of this serum from the culture medium, in contrast to the 3-4-fold induction of CNPase activity observed in the untreated cells, in cells previously treated with just 0.5 μg/ml of ML-236B (45% decrease in sterol content) no induction of CNPase occurred. Intermediate degrees of sterol depletion resulted in intermediate degress of inhibition of the CNPase induction. Moreover, the morphological expressions of glial differentiation observed in the untreated cells did not occur in the sterol-depleted cells. When exogenous cholesterol was added to the ML-236B-treated cells, the induction of CNPase, the morphological changes and the sterol/phospholipid molar ratios were preserved. The relative specificity of the effect of sterol depletion was demonstrated by the observation that the degree of sterol depletion that totally prevented the induction of CNPase had no effect on (Na^+ + K^+)-activated ATPase activity, total protein synthesis and cell viability.

The data are of particular importance for two major reasons. First, they demonstrate for the first time a specific role for sterol in glial differentiation and suggest that a critical level of membrane sterol in oligodendroglia is necessary for subsequent events in myelinogenesis. Second, the data demonstrate the particular value of a specific inhibitor of HMG-CoA reductase in the evaluation of the roles of sterol in critical events in neural development. (Supported by NIH grant RO1-HD-07464)

117.1 ADAPTIVE CHANGES OF OCULOMOTOR PERFORMANCE IN ABDUCENS NERVE PALSY, L. M. Optican*, F. C. Chu*, A. V. Hays*, D. B. Reingold*, and D. S. Zee, National Eye Institute, Bethesda, MD 20205.

We studied oculomotor adaptation in an adult with a chronic, right abducens nerve paralysis. The maximum lateral deviation of the right eye was 10° to the left of primary position. To assess changes in central innervation, movements of the viewing, normal eye (with the paretic eye covered) were recorded, using a search coil, in both non-adapted (paretic eye patched 9 days) and adapted (normal eye patched 9 days) states.

Horizontal saccades were elicited with 10° target jumps about L25°, 0°, and R25°. The saccadic pulse and step showed adaptation that depended on direction and orbital position. In the adapted state, for rightward saccades, the pulse gains were 0.6, 1.4 and 1.0 in the left, center and right fields of gaze, while for leftward saccades they were all 0.7. The pulse (P) and step (S) mismatch, ((P-S)/P), in the left, center and right fields of gaze, were 3%, 15%, and 32% for rightward, and -10%, -21%, and -30% for leftward saccades.

Initiation of pursuit was assessed in the first 130ms of smooth tracking (open-loop period) before visual feedback could influence the initial pursuit command. The duration (D) of the period of increasing eye velocity and the average eye acceleration (A) during D were measured. After adaptation, for 15°/s stimuli, A increased for leftward and rightward tracking of targets moving in the right and center fields, but did not change for the left field. For rightward tracking in the right field, A increased from 149 to 273°/s², while D stayed the same (107 to 112ms). For 15°/s stimuli pursuit velocities could reach 45°/s and occasionally the tracking response was a pendular oscillation (3Hz, ±1°) about the stimulus position. 5°/s stimuli revealed proportionately smaller adaptive changes. Thus the gain of smooth pursuit adaptively increased both for initial open-loop and subsequent closed-loop tracking.

The vestibulo-ocular reflex (VOR) gain was measured during oscillation (0.5Hz, ±5°) in darkness. In the adapted state, VOR gains for rightward and leftward slow phases were 2.8 and 1.6 with the eye in the right field of gaze and 1.2 and 1.0 with the eye in the center.

For each oculomotor subsystem, adaptation occurred for rightward movements to compensate for a lack of increase, and for leftward movements to compensate for a lack of decrease, in right lateral rectus force. The abnormal lateral rectus forces were reflected in the non-unity slope of the static position curve of the two eyes obtained with a Lancaster red-green test. Adaptive changes of vestibular, saccadic and pursuit movements depend on both orbital position and direction, and compensate for changes in orbital mechanics.

117.2 IS CEREBELLAR SACCADIC DYSMETRIA EQUAL IN BOTH EYES? T. Vilis, R. Snow* and J. Hore. Departments of Ophthalmology and Physiology, Univ. of Western Ontario, London, Ont., Canada N6A 5C1.

Optican and Robinson (1980) have postulated that, during saccades, the cerebellum acts as a "repair shop" adjusting the innervation to eye muscles so as to compensate for changes in muscle mechanical properties. Thus it is not surprising that lesions of the cerebellum (Vilis and Hore 1981, Ritchie 1976) result in saccadic dysmetria. However for the cerebellum to exert effective compensation for defects in a single muscle, it must be capable of exerting a different compensation for each muscle. A corollary of this is that lesions of the cerebellum should produce a different dysmetria in each eye.

We examined this possibility by simultaneously monitoring saccades of both eyes, using the eye coil technique. Three monkeys (two Macaca Fascicularis and one Cebus Apella) were trained to make saccades between small LED lights located on a hemispheric dome 100 cm from the monkey's head. Reversible unilateral lesions of the medial cerebellar nuclei were produced by cooling through a sheath implanted lateral to the left fastigial nucleus. In order to compensate for small differences inherent in normal eye movements or caused by inaccuracies in calibration, differences between the two eyes were calculated as a percentage change between the normal right eye/left eye ratio and that during cooling.

Differences between the eyes, of the order of 10 to 25%, were observed in the dysmetria of horizontal saccades. In all three monkeys cooling the left cerebellum produced a dysmetria in which the right eye had a larger dysmetria than the left eye for rightward saccades and a smaller dysmetria for leftward saccades. The largest difference in eye position was observed during the saccadic trajectory. This was the result of differences in the magnitude and timing of peak velocity. The difference in eye position was frequently less 50 msec after saccade termination due to differing magnitudes of undershoot or overshoot glissades in the two eyes.

These differences in saccade trajectories suggest that a different pulse command can be generated by pontine burst neurons for each muscle of yoked muscle pairs. Thus Hering's law of equal innervation would appear to be a fortuitous consequence of appropriate cerebellar compensation and not an invariant property of synaptic connections.

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Support: Medical Research Council of Canada Grant MA-5978.

117.3 A QUANTITATIVE NETWORK MODEL OF ADAPTIVE SACCADIC PERFORMANCE. M. Kuperstein & S. Grossberg. Center for Adaptive Systems, Dept. Math., 264 Bay State Rd., Boston University, Boston MA 02215

A central problem in oculomotor behavior is understanding how the eye movement system tunes itself to perform accurate saccades in development without a priori knowledge of changes in the eye or the extraocular muscles. Thus far, investigators have used control theory models to describe visual-motor transformations in the oculomotor system (1). These models have not yet explained, in neural terms, how visual-motor mapping is tuned by error correcting mechanisms during performance.

Using a different approach, we have used the network theory developed by Grossberg (2) to derive a quantitative model that explains not only saccadic error correction by also a large body of experimental data on saccadic performance. The model has evolved out of consideration of the following questions: How does the system know that an error has been made and then correct it? How are retinal signals mapped into correct saccades, independent of initial eye position? How are retinotopic signals mapped into the temporal coordination of the six extraocular muscles to achieve both direction and amplitude of saccades? How can saccades be both ballistic and achieve their goals during interruptions?

The designs that emerge suggest functional roles for parallel attentional and orienting paths; for visual-motor and motor cell types; and for the integration of glissadic and saccadic control mechanisms. These designs embody properties of networks including gradient type mapping, associative synaptic learning, short term memory, competitive interactions and external and internal feedback mechanisms.

Computer simulations show that the model is consistent with cell response curves and with data on microstimulation of brain structures. The organizing principles used in the model can be applied to other problems of sensory-motor coordination and development.

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117.4 CEREBRAL POTENTIALS PRECEDING GAZE. James A. Sharpe and R.D. Gordon Blair. Division of Neurology and Playfair Neuroscience Unit, University of Toronto, Toronto, Canada.

Surface potentials were averaged before gaze shifts in ten normal subjects. Horizontal self paced eye saccades with the head fixed and combined head and eye saccades were made at intervals over 5 seconds toward LED targets 20 degrees apart. Head-fixed and head-free gaze shifts were also performed in darkness. Scalp potentials were recorded from pre-central leads (between F3, Fz, F4 and C3, Cz, C4) and parietal leads (P3, Pz, P4), all referenced to linked ears. Potentials were digitized off-line for pre-triggered averaging on a PDP11 computer. We used a trigger moving routine to place the trigger for each epoch (N > 70) at the onset of the saccade or head movement. The amplifier time constant was 5 seconds and the high frequency cut-off was 100 Hz. Rectified cervical EMG activity was averaged in relation to head saccades. In some subjects averages were obtained from additional scalp electrodes and periorbital electrodes.

A slow negative readiness potential (RP) preceded eye saccades with the head fixed and gaze shifts with the head free, by about 1 second. The RP had a mean slope of -4.7 uv/sec. It had its steepest slope beginning some 500 msec before gaze shifts. An inconstant premotion positive potential (PMP) occurred at pre-central and parietal leads in most trials just before eye saccades with the head fixed and just before gaze shifts with the head free. These potentials were similar before gaze to targets and in darkness. We found no significant asymmetry of the RP or PMP between right and left scalp leads for gaze shifts in either direction. At pre-central and parietal leads, a positive spike potential straddled saccadic onset; at periorbital leads this spike potential was negative and larger in amplitude, suggesting an origin in orbital muscles. Volume conducted electro-oculographic potentials confounded interpretation of potentials following gaze shifts. We did not detect a premotion unilateral sharp negative potential contralateral to the direction of head or eye movements, as occurs at the pre-central area before contralateral limb movements (e.g. Shibasaki et al, EEG Clin. Neurophysiol. 49:213, 1980) but volume conducted EMG activity may have obscured such a unilateral potential before head saccades. The RP and the PMP before saccades with the head fixed, and before combined head and eye saccades were indistinguishable. This surface activity may represent cerebral motor programs that mobilize gaze.

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117.5 OCULOMOTOR REFLEXES AFTER SEMICIRCULAR CANAL PLUGGING IN CATS. J. Baker*, J. Goldberg*, R. Schor, and B. Peterson. Dept. Physiology, Northwestern Univ. Sch. of Med., Chicago, IL. 60611, and Rockefeller Univ., NY, NY 10021.

The horizontal ocular reflexes of cats were studied before and after plugging all six semicircular canals (2 cats) or the horizontal canals only (2 cats). Electro-oculographic (EOG) recordings during sinusoidal yaw rotation in the dark showed that, before plugging, all cats had a strong vestibulo-ocular reflex (VOR) and no consistent cervico-ocular reflex (COR). After plugging, the VOR was virtually absent in the cats with all canals plugged. A small phase-advanced reflex (gain = 0.07, phase = + 87 deg.) was detectable at high velocities of rotation. The adequate stimulus for this reflex was angular, not linear, acceleration. There was no VOR recovery over several months.

Cats with horizontal canals plugged were tested with whole body yaw rotation at various head pitch angles, θ, with respect to a plane perpendicular to the vertical canals. With $\theta = 0$ (near 28 deg. down from the stereotaxic plane), horizontal EOG records were similar to those from cats with all canals plugged. When the head was pitched above this null plane, a horizontal VOR appeared with gain $\cong 0.4 * \sin(\theta)$. (See figure; freq. = 0.25 Hz.) This result is close to that predicted from analysis of vertical canal contributions to horizontal eye movements (J. Goldberg et al., this meeting). Periods of compensatory and of anticompensatory eye movements occurred when the head was pitched below the null plane.

In all four cats, the VOR was partially replaced by the development of a compensatory COR, measured during body rotations with the head fixed in space. The COR appeared within a few days of plugging, and had a gain of 0.08 at 2-4 weeks. At 8-10 months the COR in one cat had developed a constant gain of 0.15 and compensatory phase from 0.1 to 2.5 Hz. In cats with all canals plugged, compensatory eye movement gain increased during active head movements. Eye movements continued when the head was braked, implicating a predictive mechanism.

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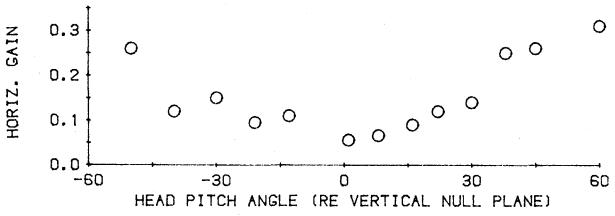

117.6 VERTICAL CANAL CONTRIBUTION TO HORIZONTAL EYE MOVEMENTS. J. Goldberg*, J. Baker*, R. Schor, B. Peterson (Spon: D. Nelson) Physiology Dept., Northwestern Univ. Med. Sch., Chicago, Il 60611 and The Rockefeller Univ., NY, NY 10021.

During horizontal head movements, vertical semicircular canals receive a small amount of stimulation depending on head elevation. We have compared horizontal eye movements produced by vertical canal stimulation in cats with plugged horizontal canals (J. Baker et al, this volume) with predictions based on canal geometry.

Rotations were applied in the earth-horizontal plane (yaw) while the animal's head was held at various pitch angles (θ) with respect to the horizontal. The effective canal stimulus thus was a function of canal orientation (stereotaxic coordinates) and of head orientation with respect to the horizontal (earth-fixed coordinates). Eye movements induced by rotation in any plane can be then described by the vector equation:

$$(1) \quad E = B*C*P*H$$

where E specifies torsional, vertical,and horizontal eye movements in stereotaxic coordinates; H specifies applied roll, pitch, and yaw rotations in earth-fixed coordinates; P transforms vectors from earth-fixed to stereotaxic coordinates; C specifies canal orientations in stereotaxic coordinates; B specifies the coupling between canals and eye movements. With C obtained by normalizing data of Curthoys et al (Acta Otolaryngol., 83:258) and averaging coplanar canals, (1) becomes:

$$E = B * \begin{vmatrix} 0.309 & 0 & -0.926 \\ 0.637 & -0.688 & 0.331 \\ 0.637 & 0.688 & 0.331 \end{vmatrix} * \begin{vmatrix} \cos\theta & 0 & -\sin\theta \\ 0 & 1 & 0 \\ \sin\theta & 0 & \cos\theta \end{vmatrix} * \begin{vmatrix} \text{roll} \\ \text{pitch} \\ \text{yaw} \end{vmatrix}$$

B is computed from (1) by assuming that normal vestibuloocular reflexes keep gaze stable in space, i.e. $P^{-1} E + H = 0$:

$$(2) \quad B = C^{-1}$$

Horizontal canal plugging reduces the first row of C to zeroes. The reduced matrix C' and matrix B predict eye movements in the plugged cat, E':

$$E' = -C^{-1} * C' * P * H = - \begin{vmatrix} 0.937\sin(\theta+62.5) & 0 & -0.937\sin(\theta-27.5) \\ 0 & 1 & 0 \\ 0.367\sin(\theta+62.5) & 0 & -0.367\sin(\theta-27.5) \end{vmatrix} * \begin{vmatrix} \text{roll} \\ \text{pitch} \\ \text{yaw} \end{vmatrix}$$

In particular, the gain of horizontal eye movements during yaw with head pitched by an angle θ (+ nose down) is given by:

$$(3) \quad 0.367\sin(\theta-27.5)$$

Least square fits to such gain measurements obtained in two cats were $0.38\sin(\theta-29)$ and $0.44\sin(\theta-36)$ for arguments less than 0. Good agreement of these data with our prediction is a direct demonstration of a functional coupling between vertical canals and horizontal eye muscles as proposed by Schultheis and Robinson on theoretical grounds (In: Physiological and Pathological Aspects of Eye Movements, in press).

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117.7 EYE POSITION DURING PERFORMANCE OF FIXATION TASKS: COMPARISON OF MONKEY AND HUMAN. Max Snodderly and Daniel Kurtz*. Eye Research Institute of Retina Foundation, Boston, MA 02114

When monkeys and humans perform the same fixation tasks, some aspects of eye position are very similar, as would be expected from the anatomical similarity of their visual systems. In particular two monkeys had intra-trial stability as good or better than human subjects. However, when the trials are run with a lighted fixation target in otherwise dark surroundings, performance of human and non-human primates is very different if between-trial measures are used. The monkeys had much greater dispersion of eye position between trials on the vertical than on the horizontal meridian for two different fixation tasks. One task was detection of the unpredictable dimming of a spot of light, the other was the unpredictable tilt of a tiny line segment. Three human subjects either had roughly equal horizontal and vertical dispersions or greater horizontal than vertical dispersions. All subjects, humans and monkeys, showed striking idiosyncratic patterns in the dispersion between trials. All subjects also showed greater dispersion of eye position between trials than within trials. In order to avoid verbal bias, one of our human subjects was trained by the same computer program that trained the monkeys with no verbal instructions. Thus the species differences are not due to differences in instructional variables. We believe they may be linked to the tendency of the monkeys to begin the trial high above the target before making a saccade to acquire the target.

117.8 LID AND EYE MOVEMENTS DURING BLINKING IN FRONTAL- AND LATERAL-EYED ANIMALS. C. Evinger, M. Shaw, C. Peck and R. Baker. Dept. Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794; Dept. Physiol. & Biophysics, NYU Med. Ctr., New York, NY 10016; Dept. Psychol., Pomona Col., Claremont, CA 91711

Blinks, like saccadic eye movements, offer many technical advantages for studying ballistic movements. Blinking is easily induced and measured. Only two muscles are involved and the motoneurons which generate the movements are readily accessible in the brainstem. HRP injections into the orbicularis oculis muscle of rabbits and guinea pigs showed that the motoneurons lay in the dorsolateral aspect of the ipsilateral facial nucleus. The motoneurons innervating the levator palpebrae muscle were situated in the dorsolateral aspect of the contralateral oculomotor nucleus in the rabbit. However, in the guinea pig these neurons lay along the lateral edge of the contralateral oculomotor nucleus, in the MLF and in the reticular formation lateral to the oculomotor nucleus.

The characteristics of upper lid and eye movements during blinking were examined in three species (human, guinea pig, and rabbit). The slope of the linear relation between downward maximum lid velocity and amplitude of lid movement was 19.8 mm/sec per mm for human, 24.4 mm/sec per mm for guinea pig, and 18.5 mm/sec per mm for rabbit. Raising the eyelid required more time than closing it. The slope of the linear relation between upward maximum velocity and lid amplitude was 7.5 mm/sec per mm for human, 15.9 mm/sec per mm for guinea pig, and 6.7 mm/sec per mm for rabbit. Experiments on the mechanical properties of the lid suggested that the difference between opening and closing resulted partially from a passive downward tension on the lid.

Upward eye movements occurred with the down phase of every blink. In humans, the upward eye movement was accompanied by adduction; in guinea pigs and rabbits, abduction. Activation of the superior rectus would produce these movements in all three species. The amplitude of the upward eye movement increased as blink amplitude increased and the velocities of these movements were comparable to those of saccadic eye movements. Since lateral eyed animals rarely make saccades when their heads are immobilized, blinking may provide an opportunity to study the central organization of vertical saccadic eye movements.

The lid moved with the eyes during downward saccades, but our EMG recordings in humans showed that the orbicularis oculis muscle was not active during eye closure associated with gaze changes. The slope of the linear relation between maximum lid velocity and lid amplitude during downward saccades was 13.7 mm/sec per mm, which was less than that for lid closure during blinks. This suggests that the passive downward tension on the lid is sufficient to produce eye closure during gaze changes.

117.9 SOME OCULOMOTOR INTERNUCLEAR NEURONS PROJECT BILATERALLY TO THE ABDUCENS NUCLEUS, R. Maciewicz, B. Phipps, W.E. Foote, Depts of Neurology and Psychiatry, Mass. General Hosp., Boston, MA 02114

Both orthograde and retrograde transport studies demonstrate an internuclear projection from the oculomotor complex (nIII) and adjacent ventral periaqueductal grey region to the abducens nucleus (nVI) and the dorsomedial central tegmental reticular nucleus (dmTRC) in the cat. To determine whether individual nIII internuclear neurons project to nVI on both sides or to both nVI and dmTRC, double retrograde labeling techniques using fluorochromes and horseradish peroxidase (HRP) were employed. Conditions of survival (two days) and fixation (3% paraformaldehyde) were optimized to decrease intercellular spread of fluorescent label. Tetramethylbenzidine was used as the substrate to demonstrate HRP, allowing simultaneous visualization of fluorochromes. HRP injection into nVI on one side resulted in the retrograde labeling of a large number of cells in the ventral periaqueductal grey and nIII on both sides of the mesencephalon. When nuclear yellow or DAPI (<50nl of 1% solution) was deposited in the nVI contralateral to the HRP injection, 9%-16% of cells labeled with HRP also contained fluorochrome, evidence that certain oculomotor internuclear neurons may have bilateral projections to nVI. Such double-labeled cells are co-extensive with single labeled cells, both within nIII and the adjacent periaqueductal grey. The frequency of double-labeling is largely independent of the amount of label injected or the degree of spread at the injection sites. In a different set of experiments, HRP was injected into nVI bilaterally and nuclear yellow deposited in dmTRC. Both tracers retrogradely labeled cells in nIII and the adjacent periaqueductal region. In these experiments double-labeled cells (containing both HRP and nuclear yellow) were only very rarely encountered, evidence that oculomotor region projections to nVI and dmTRC may arise from different subpopulations of cells. Finally, in these same cases DAPI was injected into the extraocular muscles of one eye, retrogradely labeling nIII motoneurons. No double-labeling of motoneurons occurred with either nVI or dmTRC injections, further evidence that oculomotor internuclear projections do not arise from collaterals of nIII motoneurons. These multiple label studies suggest that nIII and the adjacent periaqueductal region both contain separate populations of internuclear neurons that may differ in their brain stem connections. They provide further evidence that a subset of nIII internuclear cells project bilaterally to nVI, although the low frequency of double-labeling observed suggests the percentage of bilaterally projecting cells may be small.

117.10 OCULOMOTOR ORGANIZATION IN TWO MARINE FISH. W.J. Brunken* and W. Graf (SPON: J.I. Nelson). SUNY-Stony Brook, NY 11794, Rockefeller Univ., NY, NY 10021 and Marine Biol. Lab., Woods Hole, MA 02543.

Comparative aspects of oculomotor organization were examined in two marine fish, the elasmobranch Mustelus canis (dogfish), and the teleost Pseudopleuronectes americanus (winter flounder). Extraocular muscle orientation and kinematic characteristics in both species were comparable to lateral-eyed mammals (e.g. rabbit). In the winter flounder, the size of the horizontal eye muscles (lateral and medial rectus) was smaller than the vertical eye muscles. HRP injections into single extraocular muscles revealed the commonly described distribution of motoneurons in the oculomotor nucleus. Labeled cells were grouped around the two major fascicles of the medial longitudinal fasciculus (MLF). In P. americanus these motoneuron pools consisted of two contralateral (superior rectus, SR and superior oblique, SQ) and four ipsilateral populations (inferior oblique, IQ; inferior rectus, IR; medial rectus, MR; and lateral rectus, LR). IR neurons were located most rostrally in the oculomotor nucleus and subjacent to the ventricle. Further caudally MR and SR motoneurons were found, the latter located as a vertically oriented cell band close to the midline and extending throughout most of the length of the oculomotor nucleus. The majority of the IQ motoneurons were found in the most ventro-caudo-lateral part of the oculomotor nucleus in close proximity to and within the exiting fibers of the third nerve and more rostrally between the two major fascicles of the MLF. The most caudally located cell group in the midbrain is the trochlear nucleus, located at the ventrolateral edge of the ventricle. The abducens nucleus was found in the posterior brainstem in the neighborhood of the vestibular nuclear complex. In M. canis we found approximately the same rostro-caudal and ventro-lateral motoneuron pool distribution, however the degree of overlapping between the neuron populations is larger and MR motoneurons were located contralateral to the injected muscle. In M. canis the populations of motoneurons are approximately equal. In contrast, in P. americanus there are pronounced differences: populations of the vertical eye muscles (SR,SQ,IR,IQ) are of equal numbers (ca.60). However, the medial rectus subdivision contains only about 50% and the abducens nucleus only about 20% labeled cells as compared to the numbers counted in vertical eye muscle motoneuron pools. Differences in oculomotor organization in the studied species reflect the different living environments of these fish. M. canis is a pelagic fish, whereas P. americanus is a bottom adapted flatfish; unlike the dogfish compensatory eye movements in this fish utilize mainly the vertical eye muscles. Supported by the Grass Foundation, DFG Grant 688/1 and NIH Grant 13742.

118.1 ANGIOTENSIN II INCREASES CATECHOLAMINE SYNTHESIS IN SELECTED HYPOTHALAMIC NUCLEI. R.H. Alper, M.K. Steele and W.F. Ganong, Department of Physiology, University of California, San Francisco, CA 94143.

There is circumstantial evidence to suggest that the octapeptide angiotensin II (AII) produces some of its effects by altering the synthesis and release of central catecholamines. To date, however, this has not been reported using standard biochemical techniques in microdissected brain nuclei. The present studies were designed to determine if centrally administered AII alters the rate of DOPA accumulation (an in vivo estimate of tyrosine hydroxylase activity) in the median eminence, preoptic area, supraoptic nucleus, paraventricular nucleus, posterior pituitary and corpus striatum, regions containing terminals of dopaminergic and/or noradrenergic neurons. DOPA was measured radioenzymatically (Demarest and Moore, Endo. 106:463, 1980) in various brain regions of male rats 30 minutes after the inhibition of DOPA decarboxylase by NSD 1015 (100 mg/kg, i.p.). The rate of DOPA accumulation was increased 35 minutes after the intraventricular (ivt) administration of AII (500 ng in 2 µl 0.9% NaCl) in the median eminence (27%), preoptic area (18%) and supraoptic nucleus (37%). In a separate group of male rats this same dose of AII decreased the plasma prolactin concentration for at least 60 minutes. These data suggest that the small, but significant effect of AII on DOPA accumulation in the median eminence may reflect an increased release of dopamine from tuberoinfundibular neurons. AII did not alter catecholamine synthesis in the paraventricular nucleus, posterior pituitary or striatum. Furthermore, when AII was administered peripherally (1.5 mg/kg, s.c.), DOPA accumulation, as well as dopamine and norepinephrine concentrations, were not altered in any brain region for 35-120 minutes. These data suggest that central AII may exert some of its dipsogenic, cardiovascular, endocrine or other effects by increasing the release of catecholamines from neurons terminating in specific hypothalamic nuclei.

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118.2 EVIDENCE FOR CENTRAL PATHWAYS MEDIATING THE HYPERGLYCEMIA PRODUCED BY DOPAMINE-RECEPTOR AGONISTS IN THE RAT. S.P. Arneric*, S.A. Chow*, R.L. Webb*, R.K. Bhatnagar, J.P. Long* and L.J. Fischer*. (SPON: W. Steele) Department of Pharmacology, College of Medicine University of Iowa, Iowa City, IA 52240.

Evidence in the literature suggests that dopamine (DA) or DA receptor agonists can produce hyperglycemia in rats, monkeys or humans. The mechanisms for DA-receptor agonist-induced hyperglycemia are unclear. Previous results from our laboratory (1982, Fed. Proc. Abst. 41: 4637) indicated that apomorphine (APO) or RDS-127 [2-di-n-propylamino-4,7-dimethoxyindane]-induced hyperglycemia is blocked by DA receptor antagonists or adrenalectomy. A central mechanism for the hyperglycemia was suggested because APO or RDS-127 was more potent when given by intracerebroventricular injection than when given subcutaneously (s.c.). In this study, evidence was sought to establish whether the hyperglycemia produced by DA receptor agonists is: (1) mediated by central neural pathways (2) the result of epinephrine (Epi) liberated from the adrenal medulla (3) the result of glycogenolytic or gluconeogenic processes. Experiments were performed in conscious unrestrained male Sprague-Dawley rats (150-250 g). The animals were fasted for 18 h before the experiments unless indicated otherwise. DA receptor agonists were given s.c. and serial blood samples were withdrawn from jugular catheters.

Sectioning of the spinal cord at T_{1-2} or sectioning the greater splanchnic nerve blocked RDS-127 or APO-induced hyperglycemia. Also, RDS-127 or APO-induced hyperglycemia was abolished by adrenaldemedullation. In subsequent experiments, serum concentrations of Epi in RDS-127 or APO-treated animals were determined and found to be significantly elevated (150-220% of control). Exogenous Epi, when given s.c. in a dose that produced an equal hyperglycemic response, also elevated serum Epi concentrations to 150-220% of control. In separate experiments, it was found that the length of fasting (2, 18 or 48 h) had no significant effect on Epi-or APO-induced hyperglycemia despite a significant reduction (87-100%) of liver and skeletal muscle glycogen content following 18 or 48 h fasts. MICA (5-methoxyindole-2-carboxylic acid), an inhibitor of gluconeogenesis, blocked Epi- or APO-induced hyperglycemia in 18 h fasted rats. However, in 2 h fasted animals, MICA was ineffective in blocking APO-induced hyperglycemia.

These data suggest that stimulation of central DA-receptors by DA-receptor agonists activate descending spinal pathways, which promote the release of Epi from the adrenal medulla. Epi may then, depending on the nutritional status, facilitate glycogenolytic or gluconeogenic mechanisms to produce hyperglycemia.

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118.3 CENTRAL ADRENERGIC REGULATION OF THE HYPOTHALAMIC-PITUITARY-THYROID AXIS. C. Longserre*, C. Lynch* and L.C. Terry (SPON: J.N. Whitaker). Depts. of Neurology and Physiology, Univ. of Michigan, Ann Arbor, MI 48105.

Hypothalamic regulation of thyrotropin (TSH) is thought to be mediated by the release of both stimulatory (thyrotropin releasing hormone, TRH) and inhibitory (somatostatin, SRIF) hormones, which in turn are regulated by catecholaminergic neurons. Evidence indicates a stimulatory effect of central norepinephrine on TSH, but the role of epinephrine (EP) has not been elucidated. The purpose of the present investigations was to determine the effects of central EP depletion on episodic, cold-induced, and TRH- and SRIF antiserum-stimulated TSH secretion in freely-behaving, chronically cannulated male rats.

Male albino rats bearing chronic indwelling intra-atrial cannulae were kept on a constant light-dark cycle (12/12h with lights on at 600h) and allowed free access to food and water. Serial blood samples were removed every 15 min. for 3h beginning at 1000h. Animals were administered the norepinephrine-N-methyltransferase (NMT) inhibitors SKF 64139 (dichlorotetrahydroisoquinoline), LY 78335 (2.3-dichloro-α-methylbenzylamine), and SKF 29661 (1,2,3,4-tetrahydroisoquinoline-7-sulfonamide) or vehicle at 930h (50 mg/kg i.p.). SKF 64139 and LY 78335 cross the blood brain barrier and inhibit central EP synthesis, whereas, SKF 29661 only inhibits peripheral EP synthesis. Earlier studies showed that SKF 64139 caused a significant reduction in hypothalamic EP, but did not alter norepinephrine nor dopamine (Terry et al., J. Clin. Invest. 69:104, 1982). To assess the effects of NMT inhibitors on cold-induced TSH release, vehicle- or SKF 64139-treated animals were sampled for 1h (1000-1100h) at 26°C and exposed to 4°C for 2h. TRH (40ug) was administered to vehicle- and SKF 64139-treated animals at 1100h to assess possible pituitary effects of SKF 64139 on TRH-induced TSH release. SKF 64139- or vehicle-treated animals were also administered SRIF antiserum (0.5 ml i.v. at 1100h) to evaluate the effects of EP synthesis blockade on SRIF-antiserum-induced TSH release. Plasma TSH and serum thyroxine levels were measured by radioimmunoassay. SKF 64139 and LY 78335 caused a significant reduction in plasma TSH (335 ± 24 and 267 ± 13, respectively vs vehicle, 781 ± 30.2 ng/ml). Serum thyroxine was reduced by SKF 64139 (2.51 ± 0.13 vs 3.14 ± 0.7 ug/dl). Cold-induced TSH release was also inhibited by SKF 64139. The TSH response to TRH was partially suppressed by SKF 64139, indicating a slight pituitary effect of this agent. Pretreatment with SKF 64139 inhibited the stimulatory effect of SRIF-antiserum on TSH.

These findings support the hypothesis that the central EP system regulates TSH secretion by its influence on the release of TRH from the hypothalamus. (Supported by NIH and VA grants).

118.4 ALTERATIONS IN THE TUBEROINFUNDIBULAR DOPAMINE SYSTEM AFTER NEONATAL MONOSODIUM GLUTAMATE(MSG) TREATMENT. R. Dawson, J.J Valdes and Z. Annau. Division of Toxicology, The Johns Hopkins Univ., Baltimore, MD 21205

The arcuate nucleus of the hypothalamus is the presumed origin of the dopamine fibers that innervate the posterior and intermediate lobes of the pituitary and the palisade layer of the median eminence. MSG administration to neonatal rats results in severe neuronal necrosis within the arcuate nucleus, however, the dopaminergic cells of the arcuate nucleus appear resistant to the neurotoxic effects of low doses of MSG. The aim of the present study was to evaluate the effects of neonatal MSG administration on dopaminergic markers in the mediobasal hypothalamus and pituitary.

Female Sprague-Dawley rats were injected on postnatal days 2 and 4 with 4mg/g of MSG or saline and were killed on postnatal day 120 for neurochemical evaluation. Kinetic constants (K_m & V_{max}) were determined for the high affinity uptake of tritiated dopamine into synaptosomes prepared from either the ventral or dorsal hypothalamus. In addition, posterior pituitary dopamine levels were determined in these MSG-treated(n=12) and control (n=9) rats. In a separate series of experiments, dopamine levels were determined in the mediobasal hypothalamus, posterior pituitary and the anterior lobe of the pituitary by high performance liquid chromatography coupled with electrochemical detection.

MSG treatment resulted in a 46% decrease in the V_{max} for dopamine uptake in the ventral hypothalamus(MSG=1.16 x 10^{-13} M/ug protein/min.; Con=2.14 x 10^{-13} M/ug protein/min.) and a 50% decrease in the K_m for dopamine(MSG=1.47 x 10^{-7} M; Con=2.92 x 10^{-7} M). The loss in uptake capacity was most prominent at a dopamine concentration of 10^{-6} M which is the K_m value for the tuberoinfundibular dopamine neurons. V_{max} in the dorsal hypothalamus was unaltered by MSG treatment, however there was a 30% decrease in the K_m value in the MSG-treated rats. Posterior pituitary dopamine levels were not significantly affected by MSG treatment(MSG=1.260 ± .107 ng/lobe; Con=1.161 ± .070 ng/lobe). MSG-treated (n=6) and control (n=6) rats killed at 30 days of age did not differ in their mediobasal hypothalamic content of dopamine,norepinephrine or serotonin. Pituitary levels of dopamine in both the posterior and anterior lobes were not altered by MSG treatment. There was a substantial loss of arcuate nucleus neurons that was histologically confirmed in the MSG-treated rats. The weight wet of the anterior pituitary was significantly decreased in the 120 day old MSG-treated rats but was not reduced in the 30 day old rats. The results suggest that at the dose employed in this study there was a loss of tuberoinfundibular dopamine neurons as evidenced in the uptake experiments, however this loss is not reflected in the steady state levels of dopamine in the mediobasal hypothalamus or pituitary. (Supported by USPHS grants ES 02277 & ES 07090)

118.5 A COMPARISON OF THE EFFECTS OF SHORT TERM VERSUS CHRONIC HYPERPROLACTINEMIA ON TUBEROINFUNDIBULAR DOPAMINE TURNOVER AND PITUITARY POSTSYNAPTIC SENSITIVITY IN THE RAT, P.R. Findell*, B.R. Larsen* and B. Benson (SPON: A.E. Atwater). Department of Anatomy, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Convincing evidence demonstrates that elevations in serum prolactin (PRL) accelerate tuberoinfundibular (TIDA) dopamine (DA) turnover. It was of interest to determine whether short term and/or chronic changes in neurotransmitter turnover induced by PRL also affect pituitary postsynaptic sensitivity to DA.

Adult male Sprague-Dawley rats were made hyperprolactinemic by transplanting 3 anterior pituitary homografts under the right kidney capsule or were sham-operated. Animals were sacrificed 4 days or 8 weeks following surgery. Upon sacrifice, truncal blood was collected and the anterior pituitary and mediobasal hypothalamus (MBH) removed from each animal. Anterior pituitaries were bisected and one-half incubated in Medium 199 containing 10^{-5}M DA while its corresponding half was incubated in media containing 10^{-7}M DA. At 1, 2 and 3 hours of incubation, aliquots of media were removed for hormone determination. Serum, anterior pituitary and incubation media PRL levels were assayed using the RIA kit supplied by the NIAMDD. DA concentrations of MBH tissue extracts were determined by gas chromatographic/mass spectral quantitative analysis. Kinetic data for DA turnover were calculated from the regression coefficient of the depletiion curve generated by analyses of the MBH DA concentrations of alpha-methyl-para-tyrosine treated animals (Brodie, B.B. et al., J. Pharmacol. Exptl. Ther. 154:493, 1966).

Pituitary homografts induced a significant increase in serum PRL levels in animals sacrificed 8 weeks or 4 days after transplantation. Decreased in situ pituitary PRL levels were observed in all rats bearing homografts. DA turnover was markedly accelerated in the MBH 4 days following transplantation. However, 8 weeks following transplantation MBH DA turnover had returned to levels identical to those of sham-operated animals despite the fact that over a 5-fold elevation in serum PRL still persisted. Examination of in vitro pituitary PRL secretory profiles in response to DA indicated that no change had occurred in pituitary postsynpatic DA sensitivity 4 days after the induction of hyperprolactinemia. However, pituitaries removed from animals exposed to chronic hyperprolactinemia for 8 weeks displayed a markedly increased sensitivity to the PRL-release-inhibitory effect of DA.

In conclusion, this study provides evidence that the TIDA system does not maintain an accelerated DA turnover in response to chronic stimulation by PRL. Associated with, or possibly as a consequence of, the return of TIDA DA turnover to control levels was a markedly increased sensitivity to the PRL-inhibitory effect of DA at the in situ pituitary. Supported by NIH Grant HD-08759.

118.6 EFFECTS OF ACUTE OPIATE TOLERANCE ON PROLACTIN RELEASE. L. Grandison, Dept Physiology & Biophysics, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854 USA

In male rats tolerance develops to morphine stimulation of prolactin release. Tolerance was differentiated into an acute and a chronic phase. In this study the effects of acute tolerance on prolactin release and the possible mechanisms involved in acute tolerance were examined. Acute tolerance refers to a period of nonresponsiveness occurring 4 hrs after an initial injection of morphine SO_4 (15 mg/kg sc). Adult male rats implanted 3 days previously with jugular cannula for blood sampling were used. A second injection of morphine SO_4 4 hrs after the initial injection produced a much lower prolactin release response compared to the initial opiate response. Acute tolerance also reduced prolactin release induced by a physiological stimulus. Four hours after the administration of morphine SO_4 serum prolactin concentration was not different than that in naive rats; however, the prolactin response to restraint stress was blocked. Serotonergic neurons are believed to induce prolactin release during suckling. Pharmacological activtion of serotonergic receptors (fluoxetine 10 mg/kg plus 5 hydroxytryptophan 10 mg/kg) induces prolactin release. This pharmacological induction of prolactin release was significantly reduced by administration of morphine 4 hrs previously. Acute tolerance does not involve an effect on the pituitary. In comparison to naive rats, acutely tolerant rats had a similar pituitary prolactin concentration and content. In vitro the spontaneous release of prolactin and the TRH induced release of prolactin were the same from pituitary halves of naive and acutely tolerant rats. The response to haloperidol (0.25 mg/kg) in vivo was the same for both groups further suggesting that the pituitary was not involved. Since acute tolerance was observed in adrenalectomized rats, adrenal corticoid secretions do not mediate acute tolerance. It is concluded that a single large dose of morphine produces a period of nonresponsiveness of prolactin release observable 4 hrs afterwards. This acute tolerance has physiological consequences and involves prolactin regulatory mechanisms at the hypothalamic or extrahypothalamic level but not the pituitary. (Supported in part by PHS grants DA02395 and AM26661).

118.7 LACK OF INVOLVEMENT OF PROLACTIN IN NEUROLEPTIC INDUCED HYPERSENSITIVITY. D.C.Morgan*, P.K.Randall*, J.S.Randall*, N.A.Telford*, Y.N.Sinha*, & C.E.Finch (SPON. W. Bondareff). Andrus Gerontology Center, Univ. South. Calif., Los Angeles, CA 90007, and Scripps Res. Inst., La Jolla, CA.

In most rodents, chronic haloperidol treatment produces dopaminergic hypersensitization in the striatum. The CBA/J mouse strain fails to exhibit such hypersensitization (Severson et al, Br. Res. 210(1981)201). Prolactin has been suggested to mediate the effects of haloperidol on striatal dopamine receptor density (Ludmer & Hruska, Neurosci. Abst 6(1980)441). To determine if the dopamine receptor regulation problem in the CBA/J mice is located in the pituitary, we compared the prolactin response to haloperidol in this strain to two strains which hypersensitize following such treatments (C57BL/6J & BALB/cJ).

Male mice were administered haloperidol (2.5 mg/kg/d) in their drinking water for 0, 2, or 21 days. Plasma were collected by rapid decapitation and assayed for prolactin by radioimmunoassay. The results, shown below, revealed significant effects of strain and treatment. In control animals CBA/J prolactin levels were intermediate between C57BL/6J and BALB/cJ. All strains exhibited a 300+% increase in circulating prolactin with 2 days of haloperidol. After 21 days of haloperidol, some tolerance to the drug's effects were evident in C57BL/6J and BALB/cJ mice, while the prolactin levels continued to increase in CBA/J mice. Clearly, an impaired prolactin response in the CBA/J mice cannot explain their failure to hypersensitize following chronic haloperidol.

	BALB/cJ	CBA/J	C57BL/6J
VEHICLE	3.6+0.7 (14)	7.7+0.7 (14)	13.0+0.8 (14)
2 DAY HAL	11.3+3.0 (13)	25.4+6.3 (13)	43.0+13.9 (13)
21 DAY HAL	9.2+4.6 (12)	30.4+11.0 (13)	16.5+8.5 (13)

Plasma prolactin in ng/ml x+s.e.m. (n).

As a further test of the prolactin mediation hypothesis, male C57BL/6J mice were administered domperidone (3 mg/kg/d) as above (0, 2, or 21 days). Since domperidone does not cross the blood-brain barrier, such treatments should elevate plasma prolactin levels without blocking striatal dopamine receptors. Animals were sacrificed and their striata removed for dopamine receptor analysis with 3H-spiperone. We found no significant effect of domperidone on striatal dopamine receptor density. The 3H-spiperone bound at 0.4 nM was 681+31 fmol/mg protein for control animals, 731+23 after 2 days of domperidone, and 705+29 after 21 days. Thus, blockade of pituitary dopamine receptors without concomitant antagonism of central dopaminergic transmission is insufficient for the expression of striatal hypersensitivity. In light of these data, it seems unlikely that plasma prolactin mediates the effects of long term haloperidol on striatal dopamine receptor function.

118.8 EXCITATION OF SUPRAOPTIC PUTATIVE VASOPRESSIN NEURONS FOLLOWING ELECTRICAL STIMULATION OF THE A1 CATECHOLAMINE CELL GROUP REGION OF RAT MEDULLA. T.A. Day* and L.P. Renaud (SPON: D.W. Baxter). Division of Neurology, Montreal General Hospital and McGill University, Montreal, Canada.

Controversy exists as to the role of central noradrenergic structures in the regulation of vasopressin (VP) secretion. Thus, studies involving central injection of adrenergic agents have suggested a stimulatory role for noradrenaline (NA) yet it has also been reported that iontophoresis of NA onto neurosecretory cells of the supraoptic (SON) and paraventricular nuclei inhibits cell firing. It has recently been demonstrated that NA innervation of the SON is derived entirely from the A1 catecholamine (CA) cell group of the ventrolateral medulla (Sawchenko and Swanson, Science 214:685-687, 1981). The present study sought to examine the effect of electrical stimulation of this area of the medulla upon the activity of SON neurosecretory neurons.

The ventral surfaces of the medulla and the hypothalamus were exposed, the spinal cord transected at C7 and blood pressure monitored continuously in pentobarbitone anaesthetized male Sprague Dawley rats. Extracellular action potentials were recorded from antidromically identified SON neurons which were classified as putative VP or oxytocin cells on the basis of their spontaneous activity pattern and their response to acute increases in blood pressure induced by i.v. administration of a peripheral vasoconstrictor (cf Harris et al, Nature, 258:80-82, 1975). The ventrolateral medulla was stimulated using cathodal pulses (0.2ms duration, 25-100μA) delivered via glass coated tungsten electrodes, tip exposure 80μm. At the completion of each experiment animals were perfused and a CA histofluorescence technique used to demonstrate that stimulation sites corresponded to the A1 CA cell group caudal to the obex.

Approximately half of the "identified" SON cells tested were continuously active and unresponsive to acute rises in blood pressure; of these, 80% were unaffected by repetitive stimulation of the A1 region (20Hz, 0.5-10sec) and the remainder were inhibited. All cells that were either phasically active or inhibited by rises in blood pressure, and therefore tentatively classed as VP neurons, were found to be excited by repetitive A1 stimulation. A1 stimulation was frequently sufficient to initiate a phasic burst prematurely and to continue a burst indefinitely.

These data imply a facilitatory role for the A1 NA cell group in the regulation of the excitability of SON VP neurons. Nevertheless, it remains to be determined whether the effects of electrical stimulation of the A1 region are mediated only by activation of direct NA afferents to SON.
(Supported by the Canadian M.R.C.)

118.9 CONNECTIONS OF RAT SUPRAOPTIC NUCLEUS (SON) NEUROSECRETORY NEURONS WITH THE SUBFORNICAL ORGAN (SFO): AN ELECTROPHYSIOLOGICAL STUDY. S. Sgro*, Y.S. Siatitsas* and L.P. Renaud (SPON: B. Esplin). Montreal General Hospital and McGill University, Montreal,Canada.

Experimental observations implicate the SFO as the central site for drinking behaviour aroused by blood-borne angiotensin and an associated increase in plasma vasopressin. Recent anatomical studies at the light microscopy level indicating projections from SFO to SON prompted an electrophysiological investigation of the functional nature of this connection by examining the effects of electrical stimulation in SFO on the excitability of phasic (putative vasopressin secreting) and continuously active (putative oxytocin secreting) SON neurosecretory cells in pentabarbitone anaesthetized male Sprague Dawley rats. Single current pulses (0.1-1.0 mA) were applied to SFO and surrounding structures through stereotaxically implanted concentric bipolar electrodes. Using a ventral approach to obtain extracellular recordings from antidromically activated SON neurohypophyseal projecting neurons, we noted that SFO stimulation evoked short latency orthodromic increases (15% of cells) or decreases (50% of cells) in the excitability of both phasically and continuously active SON neurons. Similar effects observed to follow stimulation in the triangular nucleus of the septum were interpreted to be due to activation of descending SFO efferent fibres. No antidromic responses were recorded from SON neurons. Failure of the large majority of SON neurons to respond to stimulation in the adjacent hippocampal commissure and fornix suggested that the observed effects of electrical stimulation on SON neurons arose primarily from activation of SFO neurons and not from neighbouring structures.

A second series of experiments designed to confirm this interpretation employed placement of stimulating electrodes in SON with recordings from SFO neurons. In these experiments, antidromic action potentials were observed from several SFO neurons at latencies that range from 12-27 msec. Few cells in the SFO also demonstrated orthodromic responses to SON stimulation.

These data confirm the anatomical impression that SON neurosecretory neurons receive direct connections from SFO neurons. This connection would appear to engage both vasopressin secreting and oxytocin secreting neurosecretory cells. Our observations also imply that the pathway from SFO to SON cells contains both excitatory and inhibitory influences.

(Supported by The Canadian MRC).

118.10 OSMOSENSITIVITY OF SUPRAOPTIC NUCLEUS (SON) NEURONS: STUDIES UTILIZING INTRAVASCULAR PERFUSION IN THE ISOLATED RAT BASAL HYPOTHALAMUS. C.W. Bourque* and L.P. Renaud, Montreal General Hospital and McGill University, Montreal, Québec, Canada.

In-vivo recordings indicate that both oxytocin and vasopressin secreting magnocellular neurohypophyseal SON neurons respond to variations in plasma osmotic pressure. Further in-vivo investigation of the nature and location of the 'osmoreceptor(s)' is limited by a lack of mechanical stability and pharmacological flexibility. Controlled pharmacological manipulation of the neuronal environment and retention of natural and identifiable afferent and efferent connections of SON neurons can be achieved in an isolated preparation of rat basal hypothalamus perfused through the anterior cerebral artery with artificial cerebrospinal fluid (ACF). In this preparation, neurosecretory neurons display antidromic activation from the neural lobe and spontaneous activity patterns virtually identical with in-vivo recordings. Synaptic isolation, achieved by replacement of Ca^{2+} with $MgCl_2$ or NaCl with 12mM $MgSO_4$ is indicated by a reversible abolition of orthodromically induced changes in the excitability of SON cells following medial preoptic stimulation.

The present study utilizes extracellular recordings of SON cells to assess their response to an osmotic stimulus achieved by the addition of NaCl, sucrose or mannitol to the ACF to obtain pressures of 315-354 mOsm/L. Over 70% of cells respond to the osmotic stimuli by either (a) induction of activity in silent cells, (b) an increase in action potential frequency, including the appearance of phasic activity, or (c) increase in burst length and intraburst discharge frequency and shortening of inter-burst intervals among phasically active cells. During synaptic isolation 60% of cells respond to osmotic stimuli but these responses are characterized by an increased incidence of irregular activity patterns.

These observations indicate that SON cells are endogenously osmosensitive. However, reduction in the number and change in the pattern of response during synaptic isolation suggests that some form of synaptic interaction with other central neurons is an important component of the physiological response to changes in plasma osmolality.

(Supported by M.R.C.).

118.11

WITHDRAWN

118.12 MUSCARINIC RECEPTORS IN HYPOTHALAMUS: EFFECTS OF CYCLICITY, SEX AND ESTROGEN TREATMENT. K.L. Olsen, E. Edwards*, W. McNally*, N. Schechter and R.E. Whalen. Long Island Research Institute, SUNY at Stony Brook, Stony Brook, NY 11794

Steroid hormones modulate neurotransmitter activity. Possibly this interaction with neurotransmission is critical for the expression of hormonally-mediated functions. In the present study, we assessed the relationship between cholinergic binding and endocrine processes.

Cholinergic binding was measured in the preoptic area (POA) and whole hypothalamus of adult Sprague-Dawley rats (Simonsen) using the radiolabeled antagonist, 3-quinuclidinyl benzilate (^{3}H-QNB) as a ligand for muscarinic sites. Binding of ^{3}H-QNB (fmole/mg protein) was 30% higher in the POA than in whole hypothalamus from gonadectomized rats. Cyclic changes were observed in the POA with the highest binding at proestrus and the lowest binding at diestrus. Proestrus levels were 33% higher than that measured at diestrus and 37% higher than that found in POA from ovariectomized control females. In whole hypothalamus, no significant changes occurred over the estrous cycle.

Estrogen treatment increased ^{3}H-QNB binding in both the POA and in the hypothalamus of ovariectomized female rats as compared with untreated ovariectomized levels. High pharmacological doses [10 ug estradiol benzoate (EB)/120 g Bd Wt/ 48 and 24 hr before sacrifice] of EB increased binding by 42% in the POA and 17% in the whole hypothalamus, relative to castrated controls. The EB dose that is routinely used in our laboratory to activate female sexual behavior (2 ug EB/Rat/ 48 and 24 hr before testing] increased binding by 26% in the POA but had no appreciable effect in whole hypothalamus.

Male and female rats differ in their responsiveness to estrogen induction of cholinergic receptors. EB (10 ug/ 120 g Bd Wt/2 days) did not affect ^{3}H-QNB binding in either brain region of castrated males, although the level and pattern of cholinergic binding between untreated gonadectomized males and females were similar.

These data suggest that physiological changes in estrogen secretion over the estrous cycle are capable of modulating cholinergic binding in the POA, but not in hypothalamus. Pharmacological doses of EB enhance cholinergic binding in hypothalamus as well; presumably these changes are not of physiological relevance. The failure of EB to enhance binding in either the POA or hypothalamus of male rats is also consistent with the hypothesis that cholinergic mechanisms in the POA are involved in mediating sensitivity to estrogenic hormones.

118.13 DISTRIBUTION OF TESTOSTERONE 5 α-REDUCTASE IN RAT BRAIN. R. Scott,* M.E. Jurman,* and N.R. Krieger. (SPON: G.B. Koelle). Depts. of Pharmacology and Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Testosterone 5 α-reductase reduces testosterone to dihydrotestosterone (DHT). We have characterized this enzymatic activity and mapped its distribution in rat brain.

Incubations were carried out in 35 ul volumes at 37° for 90 minutes in covered glass tubes. The final testosterone concentration was 2.2 X 10^{-6}M which included ^{3}H testosterone (2.2 uCi/tube; 53 Ci/mMole). Incubation and separation of product from substrate by TLC were carried out as previously described [J. Neurochem. (1932), 38: 657]. The production of DHT with respect to time and protein concentration was linear over the intervals studied. Activity was confined to the 100,000 g pellet.

The enzyme has an apparent Km of 4.1 X 10^{-6}M and a broad pH optimum that extends from pH 6-8. For the brain regions surveyed testosterone 5 α-reductase activity varied by 10 fold. It was highest in the midbrain and pons (37-39 p moles/mg protein/h). It was ten times lower in the thalamus, caudate nucleus, frontal cortex, hippocampus, hypothalamus, olfactory tubercle and preoptic area (3-7 p moles/mg protein/h). And activity was intermediate (13-15 p moles/mg protein/h) in the cerebellum and olfactory bulb.

The steep regional variation in testosterone 5 α-reductase activity is consistent with a neuronal localization. The high activities in homogenates of the midbrain may reflect localizations for reductase that coincide with the well described midbrain dopaminergic centers.

118.14 EFFECT OF 17-ALPHA-ESTRADIOL AND 17-BETA-ESTRADIOL ON HIPPOCAMPAL EXCITABILITY IN ADULT MALE RATS. Michael R. Foy, Timothy J. Teyler and Richard M. Vardaris. Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272 and Kent State University, Kent, Ohio 44242.

Electrophysiological field potentials recorded from in vitro hippocampal slice preparations show a dose-dependent response to 17-beta-estradiol (17-β-E2) added to the incubation medium (Foy, Teyler and Vardaris, Brain Res. Bull.,1982,8(4)). In vitro hippocampal slices were prepared according to standard methods (Teyler, Brain Res. Bull.,1980,5(4)). The peak effect of the addition of 17-β-E2 occurred at a 100pM concentration; the CA1 field potential was increased by an average of 148 percent.

Treatment of the slices with the stereoisomer 17-alpha-estradiol (17-α-E2) at 100pM was done to observe whether there were any electrophysiological effects of this weak or inactive estrogen. 17-α-E2 is often used as a negative control in experiments to demonstrate both estrogen specificity of receptor-binding sites (Puca and Bresciani, Nature,1969,223) and biological responses (Harris and Gorski, Mol. Cell Endocrinol.,1978,10). A 100pM concentration of 17-α-E2 resulted in little or no effect at 10 and 20 minutes following addition of drug to the incubation medium.

The addition of a 100pM concentration of 17-β-E2 to the same slices which had been pretreated with 17-α-E2 , blocked the facilitatory response elicited by the 17-β-E2 administered alone. At 10 and 20 minutes after the addition of the β isomer to the preparation already containing the α isomer, the magnitude of the electrophysiological response was not significantly different from baseline control levels.

Since no enhancement of field potential is observed with 17-β-E2 following pretreatment of 17-α-E2, this would support the hypothesis that hippocampal modulation by gonadal steroid hormones may be due to involvement of an estrogen receptor mediated phenomena. The nature and specificity of the apparent estrogen receptor mediated electrophysiological event is not revealed in this study. The rapid appearance of an electrophysiological response to steroid hormone administration argues against the classical model of steroid hormone action involving genomic expression. This system can provide additional data concerning the nature of the mechanisms that modulate synaptic throughput in the presence of estradiol and other drugs.
(NINCDS Grant NS 16507)

118.15 AN IN VITRO METHOD FOR MEASURING ANDROGEN RECEPTORS IN CYTOSOL AND CELL NUCLEI OF MALE RAT BRAIN. M.Y.McGinnis*, M. J. Meaney*, P. J. Davis*, and B. S. McEwen (SPON:D. Micco) Dept. Anatomy, Mount Sinai Sch. Med. and The Rockefeller Univ. New York, N.Y. 10021.

Using testosterone (T) or dihydrotestosterone (DHT) as ligands, previous in vivo and autoradiographic studies have demonstrated androgen uptake in specific brain regions. We have developed in vitro cytosol and cell nuclear receptor binding assays employing the synthetic androgen ^{3}HR1881 as ligand. Unlike T and DHT, this ligand is neither aromatized nor metabolized. R1881 binds specifically and with high affinity to androgen receptors (AR) in human prostate (Walsh & Hicks,1979) and foreskin (Fichman et al.,1981). Male rats were either castrated or treated with 1mg T 1-2h prior to sacrifice. Samples from hypothalamus, preoptic area, amygdala and septum were pooled. In addition, AR binding in cortex, pituitary and seminal vesicles was measured. Tissues were homogenized in buffer and processed to obtain either purified cytosol extract or purified nuclear pellet. Nuclear receptor complexes were extracted with hypertonic KCl and extracts were incubated overnight with ^{3}HR1881 in the presence and absence of unlabeled R1881. Samples were passed through Sephadex columns and counted. Saturable, high affinity AR binding in the nanomolar range was found in both cytosol and cell nuclear preparations. Competition experiments using DHT, estradiol (E_2), progesterone (P) or corticosterone (C) in 10^{-6} to 10^{-9}M concentrations, indicated that DHT was an effective competitor for ^{3}HR1881 binding. The E_2 competed only at high concentrations. It has been shown in other tissues that both P and C compete with R1881 for cytosol receptors. The addition of 1uM triamcinolone acetonide (TAA) to the incubation medium virtually eliminated competition by P and C for ^{3}HR1881 in cytosol. Competition by P and C were low in cell nuclei even in the absence of TAA. Cytosol receptor binding was high in castrated rats whereas T-treated rats showed low levels of AR binding, presumably due to translocation to cell nuclei. In contrast, cell nuclear AR binding was high in T-treated rats and low in castrates. Administration of 1mg T to Tfm male rats resulted in very low levels of AR binding in all tissues. These results indicate that ^{3}HR1881 can be used to measure both cytosol androgen receptors, and nuclear androgen receptors via the exchange method.

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118.16 CHARACTERISTICS OF A DIURNAL VARIATION IN BRAIN ESTROGEN RECEPTOR CONTENT. M. A. Wilson* and E. J. Roy. Psychology Dept., Univ. of Illinois, Champaign, IL 61820.

We previously reported a diurnal variation in cytoplasmic estrogen receptor levels in the pooled hypothalamus-preoptic area-amygdala (HPA) of ovariectomized (OVX) female rats in the absence of circulating estrogens (Roy and Wilson, 1981). Cytoplasmic receptor levels are 20-30 percent lower in the dark than in the light in a 12:12 lighting cycle.

A role of the pineal gland was suggested by our initial findings of a reduction in brain estrogen receptor levels following melatonin injections. To assess the role of the pineal gland, cytosol estrogen receptors in the HPA of pinealectomized (PNX), OVX female rats were compared with levels in OVX controls at midlight and middark time points. Pinealectomy had no effect. Control HPA light-dark values were 18.3 ± 1.6 and 12.7 ± 0.6 fm/mg protein, respectively. Similiarly, PNX-OVX receptor levels were 18.5 ± 1.3 versus 12.8 ± 0.7 fm/mg protein. These results indicate that there is no direct role of the pineal gland in the control of light-dark differences in cytosol estrogen receptor amounts.

Separate experiments examined possible regional differences in the rhythm. The mediobasal hypothalamus, preoptic area, and amygdala were assayed separately for light-dark differences in cytoplasmic estrogen receptor levels. Midlight receptor levels in all three areas showed a 20-25 percent increase over middark levels in the same area, although the effect in the amygdala did not attain statistical significance. These results demonstrate that light-dark differences in cytosol estrogen receptor content exist in each of these three estrogen concentrating areas.

In a third study, we examined possible sex differences in the light-dark changes in cytosol estrogen receptor levels. Pooled HPAs of castrated male rats were assayed for estrogen receptors at midlight and middark. Receptor levels were 14.1 ± 2.6 and 13.8 ± 3.4 fm/mg protein for light and dark, respectively. Thus, no light-dark differences in estrogen receptor levels were found in castrated male rats.

To summarize, a diurnal variation in cytoplasmic estrogen receptor levels exists in the hypothalamus, preoptic area, and amygdala of female, but not male, castrated rats. This variation is unaffected by pinealectomy.

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118.17 INTERRUPTION OF ESTROUS CYCLICITY BY AN IRON CHELATOR IN THE RAT BRAIN. Joanna M. Hill* (SPON: P.D. MacLean). Laboratory of Brain Evolution and Behavior, National Institute of Mental Health, Bethesda, MD 20205.

In earlier studies we found the following indications that brain iron plays a role in the control of neuroendocrine mechanisms: (1) it was shown both histochemically and quantitatively that the amount of pallidal and nigral iron is significantly greater in the female than the male; (2) pallidal and nigral iron almost doubles during proestrus and (3) shows another rise during early pregnancy; (4) iron is histochemically located in many brain sites known to be estrogen sensitive and/or having an influence on pituitary regulation. It is of particular note that iron occurs in high concentration in the organum vasculosum of the lamina terminalis (OVLT) and in the tanycytes of the ventromedial hypothalamus and median eminence (Hill, J.M., Neurosci. Abstr. 6: 131, 1980; Neurosci. Abstr. 7; 219, 1981; also Thesis, 1981.

The present study was designed to learn whether or not intraventricular injection of an iron chelator in the region of the ventromedial hypothalamus and median eminence would affect the estrous cycle. Vaginal smears of adult female rats were taken daily. After establishing the vaginal cyclicity pattern, 10 animals received 3.0 µl of a 0.1% solution of the iron chelator deferoxamine mesylate. Another 10 females received an equal amount of saline in the same region. Vaginal smears were taken daily for two or more weeks following the injection. Within two days after treatment 8 of 10 animals of the experimental group and 2 of 10 controls were found to be in constant diestrus ($p<0.01$, Fisher's Exact Test). Duration of anestrous was the equivalent of 2 to 4 cycles ($\bar{X}=3$) after which all animals recovered normal cycling patterns. All animals were sacrificed during diestrus. Examination of the brains with the diaminobenzidine intensified Perl's reaction for iron revealed suggestive paling of the tanycytes of the median eminence in animals treated with the chelator.

This study demonstrates that the intraventricular administration of the iron chelator, deferoxamine mesylate, results in a temporary disruption of the estrous cycle. The findings support the hypothesis that some form of iron is important in the neuroendocrine regulation of the estrous cycle.

118.18 EFFECT OF STRESS ON LEVELS OF PITUITARY CYCLIC AMP AND PLASMA HORMONES. G.J.Kant, J.L.Meyerhoff, B.N.Bunnell, E.H.Mougey*, D.R.Collins*, L.L.Pennington*, C.C.Kenion*, G.C.Driver*, W.L.Gamble*, C.B.Wormley*, L.Landman-Roberts* and T.Eggleston*. Dept. Med. Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012.

We have previously demonstrated that application of a single stressor for 15 min is sufficient to elevate pituitary levels of cyclic AMP in male rats in vivo (Neurosci. Abs. 7, 869, 1981). The increase in pituitary cyclic AMP appeared to be proportional to the severity of the stressor as judged by plasma hormonal response. We have now examined the effects of repeated stress on pituitary cyclic AMP and plasma hormonal response .

Three groups (A,B,C) of rats were studied for each of 5 stressors (forced running, immobilization, footshock, cold, and saline-injection). Groups A and B were exposed to 15 min. stress sessions daily for 10 days. Each rat was exposed to only one type of stressor. Group C was not stressed. On the 11th day group A rats were sacrificed without an additional stress session; B and C groups were stressed for 15 min. and then immediately sacrificed. An additional control group (D) was never exposed to stress and was sacrificed on the 11th day also. Rats were sacrificed by high-power focussed microwave irradiation. Blood was collected in heparinized beakers and plasma was stored at -20C until assayed for plasma hormones. Pituitaries were removed and sonicated in 1 ml of sodium acetate buffer. Supernatants were stored at -70C until assayed for cyclic AMP by radioimmunoassay.

Cold and saline-injection 15 min. sessions were ineffective stressors judged by neuroendocrine response. In rats exposed to forced running, immobilization, or footshock, both B and C groups had significantly elevated pituitary cyclic AMP levels compared to groups A and D which were not subjected to stress on the 11th day immediately prior to sacrifice. Habituation to the stressors developed after 10 daily sessions as shown by diminished pituitary cyclic AMP response in the repeated-stress group (B) as compared to the single stress group (C). Groups A and D had similar pituitary cyclic AMP levels suggesting that 24hrs following stress pituitary cyclic AMP levels are back to baseline. Forced running data are shown below. Pit cAMP (picomoles/mg wet weight); Prolactin (ng/ml).

Group	days1-10	day 11	Pit cAMP	Prolactin
A	stress	no stress	1.04±.12	22.7±12.8
B	stress	stress	1.80±.30	27.6±12.0
C	no stress	stress	4.92±1.6	92.7±6.3
D	no stress	no stress	1.02±.07	4.7±1.0

118.19 EVIDENCE FOR HYPOTHALAMIC ASYMMETRY IN NEURAL CONTROL OF THE GONADS. D.M. Nance, J.P. White* and W.H. Moger*. Depts. of Anatomy and Physiology/Biophysics, Dalhousie Univ., Halifax, N.S. B3H 4H7.

Increases in serum FSH are observed following hemigonadectomy (Hemi-x) of prepubertal male rats. This endocrine response is blocked by unilateral hypothalamic hemi- islands (HH-Is) which are located ipsi-, but not contralateral, to the Hemi-x (Neurosci. Abst. 6:189.8, 1980). This possible neurally mediated pheomenon in males appears to be specific to HH-Is on the right side of the brain (Nance and Moger, Brain Res. Bull., 1982). We tested the effects of HH-Is on a related endocrine challenge in prepubertal female rats. Ovarian compensatory hypertrophy (OCH) was determined 1 wk following HH-Is on the left side and Hemi-x on the ipsi- or contralateral side.

	(N)	1st (Ovarian Wt.)	2nd
Left HH-Is Contralat-Hemi-x	(9)	7.5±0.6	11.6±0.5*+
Left HH-Is Ipsilateral-Hemi-x	(9)	7.7±0.3	9.5±0.2
Sham HH-Is Unilateral-Hemi-x	(8)	7.5±0.6	12.7±0.9*
Sham HH-Is Sham Hemi-x	(8)	--	9.1±0.3

*P < 0.05, vs. Sham HH-Is, Sham Hemi-x.
+P < 0.001, vs. Left HH-Is, Ipsilateral Hemi-x.

OCH was blocked in the ipsi- but not the contralateral Hemi-x group, results which suggest a neural contribution to this endocrine reflex. A potential asymmetrical involvement of the hypothalamus in OCH of the prepubertal rats was suggested by the further observation that this phenomenon could not be demonstrated in rats given HH-Is on the right side and Hemi-x on the ipsi- or contralateral side.

	N	1st (Ovarian Wt.)	2nd
Right HH-Is Contralateral Hemi-x	(8)	6.9±0.3	9.6±0.8
Right HH-Is Ipsilateral Hemi-x	(9)	6.4±0.6	10.4±1.0

These data suggest that in addition to a possible direct neural contribution to endocrine feedback, the two-halves of the hypothalamus may differ in terms of their sensivitity to various components of endocrine control. The extent to which these hypothalamic differences are sex, age and strain dependent are currently being examined. Supported by Canadian MRC Grants # MA-6807, MA-6956 and MA-5401

119.1 FOREBRAIN PROJECTION OF NUC. COMMISSURALIS: AN ELECTROPHYSIOLOGICAL STUDY OF PRESUMED A2 NORADRENERGIC NEURONS. S.D. Moore* and P.G. Guyenet., Dept. of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908.

The retrograde transport of HRP was combined with the catecholamine (CA) fluorescence method of Furness et al. (Histochem J., 1977 9, 745) to determine the proportion of nuc. commissuralis noradrenergic (NE) cells projecting to or through the median forebrain bundle area (MFB). Up to 80% of all CA-containing neurons visualized with this technique were found to project to or through the MFB. Moreover, CA-fluorescent cells represented 90% of all retrogradely labelled neurons. Since the CA-fluorescence technique used labels only NE neurons but not E neurons, the present data indicate that the A2 noradrenergic group constitutes at least 90% of all nuc. commissuralis neurons projecting to or through the MFB area.

Nuc commissuralis neurons projecting through the MFB area were identified with single-unit recording by antidromic (AD) activation in chloral hydrate (CH) anesthetized rats (77 cells). These cells had a conduction velocity of 0.46 m/sec and a firing rate of 0 to 14 spikes/sec. Their discharges were not correlated with the heart rate and were unaffected by somatic stimulation (sciatic nerve). Vagus nerve stimulation produced an orthodromic activation (latency 28-38 msec) followed by a long-lasting inhibition (up to 200 msec). Locus coeruleus NE neurons in the same preparation were similarly affected by vagal stimulation but were in addition powerfully activated by sciatic nerve stimulation. The spontaneous discharge rate of MFB-AD-activated nuc. commissuralis neurons was inhibited by the i.v. administration of the $\alpha 2$ agonist clonidine (means ± SEM of ED 50's for 6 cells: 28 ± 4 µg/kg). The effect of clonidine was antagonized by the i.v. administration of the $\alpha 2$ antagonist yohimbine (1 mg/kg).

In conclusion, the above anatomical and electrophysiological data indicate that the vast majority of nuc. commissuralis neurons AD-activated from the MFB belong to the A2 noradrenergic cell group. In the CH-anesthetized rat, A2 NE cells present a number of electrophysiological characteristics in common with those of locus coeruleus NE cells (A6) with one important difference: A2 neurons are activated by visceral sensory inputs only, while A6 cells are activated by both visceral and somatic sensory inputs. Supported by a grant-in-aid from the American Heart Association Virginia Chapter, HL 28785 and NIHTGM 0726705.

119.2 RECURRENT INHIBITION OF SYMPATHETIC PREGANGLIONIC NEURONS BY EPINEPHRINE. Donald N. Franz, Parley W. Madsen, and Chaichan Sangdee* Department of Pharmacology, University of Utah, Salt Lake City, Utah 84132.

Our previous findings that enhancement of descending intraspinal transmission to sympathetic preganglionic neurons (SPGNs) produced by phosphodiesterase (PDE) inhibitors is prevented by clonidine and is greatly prolonged by alpha-2 receptor antagonists suggested that a local negative feedback system operates through alpha-2 receptors to limit increases in the excitability of SPGNs (Neurosci. Lett. 28:211, 1982). The present study characterized the transmitters and receptors in the negative feedback pathway. Sympathetic discharges were evoked (0.1 Hz) by stimulation of descending pathways in the cervical dorsolateral funiculus and were recorded from upper thoracic preganglionic rami in spinal cats.

Intravenous injection of 1 mg/kg of the PDE inhibitors, isobutylmethylxanthine (IBMX) or RO 20-1724 (RO), alone rapidly and markedly enhanced intraspinal transmission to SPGNs to 155-210% within 20 min, thereafter declining to control levels by 2-3 hr. Following pretreatment with yohimbine HCl (0.5 mg/kg), the typical enhancement by the PDE inhibitors did not terminate but continued to increase for more than 5 hr. Depletion of central EPI stores by a selective PNMT inhibitor, LY 134046 (20 mg/kg), gradually enhanced intraspinal transmission to 150-200% at 4 hr (Neurosci. Abstr. 7:820, 1981); subsequent administration of IBMX or RO produced typical rapid enhancement which did not decline but continued to increase for up to 4 hr. The nicotinic receptor antagonist, dihydro-beta-erythroidine (2 mg/kg), also prevented the early decline of enhancement produced by both IBMX and RO, but atropine was ineffective.

The nearly identical abilities of an alpha-2 antagonist, depletion of EPI, and a nicotinic antagonist to prevent the early termination of enhancement by the PDE inhibitors suggests that recurrent inhibition of SPGNs is mediated through axon collaterals which activate nicotinic receptors to release EPI from EPI terminals. Like clonidine, EPI appears to activate postsynaptic alpha-2 receptors that are negatively coupled to adenylate cyclase to reduce intraneuronal cyclic AMP levels and limit SPGN excitability.

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119.3 SELECTIVE INHIBITION OF PNMT PREVENTS DEPRESSION OF SYMPATHETIC PREGANGLIONIC NEURONS (SPGNs) BY METHYLDOPA. Parley W. Madsen and Donald N. Franz. Department of Pharmacology, University of Utah, Salt Lake City, Utah 84132.

The possibility that the vasodepressor effect of methyldopa (MD) is produced by methylepinephrine (mEPI) instead of methylnorepinephrine (mNE) is supported by studies showing that mEPI is highly bound to central alpha-2 receptors (Goldberg et al., Eur. J. Pharmacol. 69:95, 1981) and depresses SPGNs by potent activation of alpha-2 receptors (Guyenet and Stornetta, Brain Res. 235:271, 1982). We previously reported (Neurosci. Abstr. 7:820, 1981) that sympathetic discharges, recorded from upper thoracic preganglionic rami and evoked by activation of spinal reflex or descending pathways in the cervical dorsolateral funiculus in spinal cats, are only modestly enhanced 3 hr after i.v. infusion of 150 mg/kg of MD. However, a subsequent 5 mg/kg dose of reserpine, which alone causes no depression, produces prompt, marked depression of transmission through each pathway which is antagonized by yohimbine, suggesting that reserpine releases an active metabolite of MD to depress SPGNs by activating alpha-2 receptors.

These experiments were repeated in spinal cats after selective depletion of central EPI stores and blockade of mEPI synthesis by a selective PNMT inhibitor, SKF 64139 (Fuller et al., Biochem. Pharmacol. 30:1345, 1981). SKF 64139 (20 mg/kg) was injected 4 hr prior to 150 mg/kg of MD, at which time an additional 10 mg/kg was given. Transmission through the intraspinal pathway was gradually enhanced to 500% of control by 3 hr. In marked contrast to the previous studies, the typical depression produced by 5 mg/kg of reserpine 3 hr after MD was completely prevented by the PNMT inhibitor. This effect was not due to blockade of alpha-2 receptors by the inhibitor because clonidine (25 ug/kg) produced prompt depression of both pathways which was antagonized by yohimbine (0.5 mg/kg).

The ability of PNMT inhibition to prevent depression of SPGNs by MD supports the proposal that mEPI released from bulbospinal EPI pathways may be the functional inhibitory metabolite of MD at this site; release of EPI may also contribute. The profound enhancement of intraspinal transmission by MD may be due to release of NE and/or mNE from excitatory NE pathways that innervate SPGNs.

(Supported by NIH grants HL-24085 and GM-07579.)

119.4 IMMUNOHISTOCHEMICAL ANALYSIS OF THE CENTRAL CONNECTIONS OF THE VAGUS NERVE IN THE RAT: NEUROTENSIN-IMMUNOREACTIVITY WITHIN THE NUCLEUS TRACTUS SOLITARIUS AND VAGAL MOTOR NUCLEI. Gerald A. Higgins*[1,2], Gloria E. Hoffman [3], Susan Wray[3] and James S. Schwaber[1]. [1]DuPont Central Research and Development, Glenolden PA 19036, [2]Dept. Anatomy and Neurobiol., Univ. Vermont Coll. Med., Burlington, VT 05405 and [3]Dept. Anatomy, Univ. Rochester Sch. Med. Dent., Rochester NY 14642

Central administration of neurotensin (NT) or its congeners produces profound alterations in autonomic function, including changes in blood pressure and gastric acid secretion. Recent immunocytochemical studies have identified NT-immunoreactive and other neuropeptide-containing elements within the nucleus tractus solitarius (NTS), dorsal motor nucleus of the vagus nerve (DVN) and the nucleus ambiguus (NA). In order to examine in detail the distribution of NT and other neuropeptide-containing cells and fibers within the NTS, DVN and NA, and determine their relationship to anatomically-defined components of the vagus nerve, we have combined immunohistochemical staining with retrograde and transganglionic labeling of the central processes of the cervical vagus nerve.

Immunohistochemical staining for NT, cholecystokinin (CCK), metenkephalin (M-ENK), somatostatin (SOM), and vasoactive intestinal polypeptide (VIP) was combined with retrograde cellular labeling by HRP or fluorescent tracers in the same tissue sections. Transganglionic labeling was visualized on alternate sections of the HRP series using TMB as the chromagen. Several animals received intracisternal injections of colchicine (70µg; 48 hours survival) to intensify peptide staining within cell bodies.

NT-immunoreactive cells and fibers are heavily concentrated within the dorsomedial medulla. Caudal portions of the DVN and lateral portions of the nucleus at the level of the obex are densely innervated by NT fibers which appear in close apposition to retrogradely-labeled motoneurons. NT cell bodies and fibers are topographically distributed within the NTS; cells are located dorsal and medial to the tractus solitarius (TS) in a region heavily innervated by the vagus nerve, while fibers are present in greatest concentration dorsal to the medial aspect of the DVN, a region sparsely innervated by the vagus. Scattered NT fibers are also present within the NA. Other neuropeptides are concentrated in the NTS and DVN, with CCK, M-ENK and SOM-immunoreactive fibers surrounding vagal motoneurons and CCK, M-ENK, SOM and VIP fibers present within subdivisions of the NTS including the TS.

The close association of NT-containing cells and fibers to vagal motoneurons and sensory elements may underly the autonomic effects observed following central NT administration.

119.5 REGIONAL DISTRIBUTION OF ANGIOTENSINOGEN IN GENETIC HYPERTENSIVE RAT BRAIN. D. P. Healy* and M. P. Printz* (SPON: M. E. Baker). Division of Pharmacology, University of California, San Diego, La Jolla, CA 92093.

The brain renin-angiotensin system (BRAS) has been implicated in the development and maintenance of hypertension in the Okamoto-Aoki strain of genetic hypertensive rat (SHR). Since angiotensinogen, the precursor for angiotensin I (ANG I), may be rate-limiting in the synthesis of ANG II centrally (as it is in the periphery), changes in the level of angiotensinogen may indicate an alteration in the activity of the BRAS. Therefore, to examine the involvement of the BRAS in hypertension, angiotensinogen was measured in discrete brain areas of developing and established hypertensive SHR and their normotensive controls, Wistar-Kyoto rats (WKY).

Blood pressure was measured at 7 and 16 wks of age using the indirect tail-cuff method. The animals were then decapitated and the brains rapidly removed and frozen. Thick frozen sections were taken coronally throughout the brains and tissue punches of 38 brain areas were collected using the stereomicroscope. Angiotensinogen was measured as previously described (Lewiki, J., et al., Brain Res., 158:359-371, 1978).

The blood pressure of the 7 week old SHR group was slightly elevated relative to the WKY group (158 ± 2 vs 123 ± 2 mmHg), whereas at 16 wks of age the difference in pressure was greater (194 ± 3 vs 141 ± 3). Angiotensinogen levels were elevated throughout the brain of the 7 week SHR, relative to WKY. The greatest increases were seen in the caudate nucleus, amygdaloid area, preoptic area, median raphe, A2 area, subfornical organ, area postrema and organum vasculosum laminae terminalis. At 16 wks of age the elevation of angiotensinogen levels in SHR relative to WKY was less marked, but was significantly higher in the caudate, preoptic area, supraoptic nucleus, lateral hypothalamus, substantia nigra and periaqueductal grey.

Interestingly, the increases in angiotensinogen were seen in both developing and established hypertensive rats. Also, the areas affected were areas largely associated with cardiovascular control or neuroendocrine regulation. Therefore, these results add further support to the claim that an alteration in the regulation of the BRAS may contribute to the development or maintenance of the elevated blood pressure in SHR.

These studies were supported by a grant from the National Heart, Lung and Blood Institute, HL 25457.

119.6 DIFFERENTIAL CARDIOVASCULAR AND EEG RESPONSES RELATED TO SELECTIVE ACTIVATION OF NEUROTRANSMITTER RECEPTORS IN THE NUCLEUS TRACTUS SOLITARIUS. W.T. Talman, L. Criscione*, R. Laguzzi* and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

The nucleus tractus solitarius (NTS), the site of termination of visceral afferents of the IXth and Xth cranial nerves, mediates and integrates the reflex cardiovascular and noncardiovascular responses to cardiopulmonary and other visceral afferent stimulation. To determine whether two of these responses, viz., the baroreceptor reflex and the modulation of EEG rhythm by the NTS, are mediated by different neuronal systems in NTS, we have studied the responses of arterial pressure and EEG to four neurotransmitters (L-glutamate - L-glu, acetylcholine - ACh, serotonin - 5-HT, and substance P - SP) present in the NTS. Adult male Sprague Dawley rats were anesthetized with halothane or chloralose and cannulated both for recording arterial pressure and heart rate and for i.v. delivery of phenylephrine. The baroreceptor reflex was tested by determining the maximal reflex bradycardia following pressor (40-50 mm Hg rise in arterial pressure) doses of i.v. phenylephrine. Agents were microinjected (0.1 µl) through glass micropipettes stereotaxically placed unilaterally or bilaterally into the NTS. L-glu, ACh, and 5-HT, but not SP, produced dose-dependent hypotension with threshold doses of 30 pmoles, 10 pmoles and 100 pmoles, respectively, and comparable maximal falls of arterial pressure ranging from 33-39 mm Hg. On the other hand, L-glu, 5-HT, and SP, but not ACh, elicited hippocampal theta activity in the EEG. The cardiovascular effects of L-glu, ACh, and 5-HT could be further differentiated by the effects of bilateral microinjections of appropriate antagonists (glutamate diethylester-GDEE, atropine-Atr, and metergoline-Met, respectively) on the baroreceptor reflex. GDEE totally blocked the baroreceptor reflex as well as the cardiovascular and EEG effects of 5-HT and the cardiovascular effects of ACh. The baroreceptor reflex was decreased by 45% by Atr but was not affected by Met. Neither Atr nor Met blocked the responses to L-glu. Thus, EEG and cardiovascular changes can be elicited by activation of L-glu and 5-HT receptors in NTS while activation of ACh receptors produces only EEG changes. Different physiological functions mediated by the NTS can be dissociated and may represent distinct neurochemical mechanisms.

(Supported in part by NIH Grants HL 18974, NS 03346, and an Established Investigatorship awarded to WTT by the American Heart Association.) Visiting Scientists: LC, CIBA Geigy, Basel, Switzerland; RL, INSERM U_3, Paris, France.

119.7 STUDIES ON INTRAVERTEBRAL ARTERIAL INJECTION (IVA), VENTRAL MEDULLARY SURFACE APPLICATION (VMS) AND NUCLEUS AMBIGUUS (NA) MICROINJECTION OF NICOTINE (N) IN ACUTELY DECEREBRATED DOGS. K.M. Wu* and W.R. Martin, Department of Pharmacology, University of Kentucky College of Medicine, Lexington, KY 40536.

A series of IVA, VMS and NA microinjection experiments were conducted to investigate the effects of N on blood pressure (BP), heart rate (HR), respiratory rate (RR) and minute volume (MV) in acutely decerebrated dogs. (1) 0.05-5µg/kg of N injected into the cannulated left vertebral artery in 1 mℓ volume did not significantly change BP, HR, RR and MV (6 experiments). (2) 50µg/50µℓ of N applied to the VMS (AP=-10, L=0 mm) produced a mecamylamine (M) (100µg/5µℓ at the same VMS site) antagonizable bradycardia, hypotension and respiratory depression.

	Control	Saline	N	N + M	
HR	126±19(7)	128±18(7)	90±20(7)*	125±21†	(5)
MBP	143±10(7)	144±10(7)	91±7 (7)*	134±10*†	(5)
RR	23±2 (5)	23±3 (5)	8±2 (5)*	22±1†	(4)
MV	0.431±0.055(5)	0.433±0.062(5)	0.152±0.045*(5)	0.432±0.045†(4)	

Significant difference ($p<0.05$): * M or N vs saline, † N vs. M. Values are mean ± SEM. HR:beats/min, MBP:mean BP mmHg, RR:breaths/min, MV:ℓ/min/kg.

(3) 5µg/0.5µℓ of N microinjected into the area of NA produced hypotension accompanied by bradycardia or tachycardia. These effects can be antagonized by M (10µg/µℓ) or hexamethonium (C6) (10µg/0.5µℓ) when administered through another barrel of the multibarreled chemotrode.

	Saline	N	N + M	N + C_6
ΔHR	0.1±0.4(36)	-15.9±3.2*(23)	2.3±1.5†(10)	2.1±5.2†(9)
ΔBP	0.2±0.6(36)	-15.5±3.5*(23)	3.2±2.3†(10)	1.9±2.9†(9)
ΔHR		19.2±3.8*(13)	-3.2±1.4†(8)	
ΔBP		-16.2±1.9*(13)	2.9±2.7†(8)	

Significant difference ($p<0.05$): treatments vs. saline (*), vs. N (†). ΔHR, ΔBP are changes in HR and BP respectively when compared to saline.

The effects of N following VMS administration on BP and HR have been observed by others, however respiratory depression was not observed in chloralose-urethan anesthetized (Dev and Loeschcke., Pflüg. Arch. 379:19, 1979) and pentobarbital anesthetized atropinized cats (Feldberg and Guertzenstein., J. Physiol. 258:337, 1976). These results suggest that nicotinic receptors exist in the VMS and in the vicinity of NA and that the central nervous system effects of N on BP, HR and respiration are mediated through multiple sites of action. (Supported by the Tobacco and Health Research Institute of the University of Kentucky).

119.8 ANATOMIC RELATIONSHIPS BETWEEN VAGAL PREGANGLIONIC NEURONS AND AMINERGIC AND PEPTIDERGIC NEURAL SYSTEMS IN THE BRAINSTEM OF THE RAT. P.E. Sawchenko and L.W. Swanson. The Salk Institute, La Jolla, CA 92037.

A combined retrograde transport-immunofluorescence method has been used to delineate the relationships between cells and fibers that cross-react with antisera against oxytocin (OXY), vasopressin (VAS), substance P (SP), dopamine-β-hydroxylase (DBH), or serotonin (5HT), and vagal preganglionic neurons, which were labeled by injecting true blue (50-100 nl of a 5% suspension) beneath the sheath of the cervical vagi, bilaterally. Pretreatment with colchicine (50-100 µg, icv) was used in some experiments to enhance immunofluorescent staining of cell bodies.

A small population of retrogradely-labeled cells (30-60 per brain) at the rostral pole of the dorsal motor nucleus of the vagus (DMX) also stained positively with anti-DBH, confirming the existence of vagal preganglionic neurons that may release norepinephrine along with, or instead of, acetylcholine (Ritchie et al., Anat. Rec., 199:213A, 1981). Through the remainder of the dorsal vagal complex, and in the ventral medulla, DBH-, SP-, and 5HT-immunoreactive cells were often found in close apposition to retrogradely-labeled cells, but no additional doubly-labeled cells were detected.

Fibers stained using each antiserum were found in close association with specific groups of preganglionic neurons. A moderate number of OXY-stained fibers was found throughout the rostrocaudal extent of the DMX. At about the level of the obex, immunoreactive fibers were not distributed evenly, but rather were concentrated in the medial and lateral tips of the nucleus. Only widely scattered OXY-stained varicosities were found in association with retrogradely labeled cells in and around the nucleus ambiguus (NA). The distribution of VAS-stained fibers in these regions is quite similar, although the density of this input appears far less than that provided by oxytocinergic fibers. A rather heavy 5HT-stained input was associated with labeled cells in the DMX, especially in its medial aspect. Somewhat fewer 5HT-stained fibers were localized in the NA, although some retrogradely-labeled cells ventrolateral to the NA, proper, were encapsulated by arrays of 5HT-stained varicosities. SP-positive fibers formed an extremely dense plexus throughout much of the length of the DMX, while the NA was very sparsely innervated.

These results suggest that selective modulation of vagal mechanisms in the periphery may be achieved both by the differential release of neuroactive substances other than acetylcholine from preganglionic terminals, and by the differential innervation of functionally distinct groups of preganglionic neurons by a variety of chemically specified fiber systems from within the CNS itself.

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119.9 OPPOSITE EFFECTS EXIST IN CARDIOVASCULAR CONTROL AFTER CENTRAL AND PERIPHERAL ADMINISTRATION OF ENKEPHALIN, VASOPRESSIN, CALCITONIN AND PROLACTIN. G. DELBARRE, B. DELBARRE and J.P. BEUGRAS*
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Intracerebroventricular injection (ICV) of alpha sympathomimetic drugs produce hypotension. On the other hand, cholinomimetic drugs induce hypertension. The different results obtained by peripheral and central administration may be explained by differences in receptor function or by a possible feed back effect of circulating neurotransmitters modulating the brain centers. Hence, an attempt has been made to delineate the peripheral and central action of peptides involved in blood pressure regulation and pain sensitivity. In the rat, D. ALA MET. ENKEPHALIN ICV (0,4 - 1,6 µg/rat) induces hypertension and IV hypotension. Vasopressin, may be released by enkephalin, ICV (0,5 mU - 1,5 mU) induces hypotension and IV (0,5 - 1,5 mU) induces hypertension. Calcitonin (5 mU - 20 mU) ICV induces hypotension and IV (5 mU - 20 mU) hypertension. Prolactin, released by calcitonin ICV (2 - 5 U) induces hypertension and IV (20 - 40 U) hypotension. These peptides have opposite effects after central and peripheral administration as well as neurotransmitters.

119.10 BLOOD PRESSURE AND LOCOMOTOR ACTIVITY IN SPONTANEOUSLY HYPERTENSIVE RATS(SHR's) AFTER 6-OHDA TREATMENT. M.F. Callahan, M. Beales, G. Oltmans, S.A. Berenbaum*, P.E. Meyers*, G. Pullen* and T. Hansen*. Depts. of Pharm., Psych., Anatomy, and Physio., University of Health Sciences/Chicago Medical School, N. Chicago, IL 60064

Numerous investigations have suggested that abnormalities in CNS catecholamines norepinephrine(NE), epinephrine, and dopamine (DA) may be causally related to the development of hypertension in the SHR. The purpose of our present studies was to study the relative role of central NE and DA in this process and to observe activity levels in these animals.

Lesions of central CA systems in 5 week old SHR pups were made by infusing 250 µg of the neurotoxin 6-hydroxydopamine (6-OHDA) into the lateral ventricle (6-OHDA group). To protect NE neurons, another group of SHR pups was administered des-methyl imipramine prior to 6-OHDA (6-OHDA·DMI group). A third group received an intracerebral infusion of 6-OHDA (8µg) in the area of the ascending NE fibers (VB group). Control SHR pups (SHR-VEH group) received vehicle infusions. Blood pressures (tail cuff) were determined pre-operatively and 10, 20 and 34 days post-op. Activity levels (30 minutes) were measured 5 weeks post-op. Animals were sacrificed 2 months post-op and the lesion effects on brainstem (BS), telencephalon (TELE) and hypothalamus (HT) NE and DA levels were assessed.

Preoperative blood pressure (bp) was 150±4 ($\bar{X}$±S.E.M.). All but one lesion group (6-OHDA group) showed a significant increase in bp by 34 days post-op (6-OHDA·DMI=192±5; VB=188±6; SHR-VEH=200±12). The 6-OHDA group showed a small non-significant increase from baseline at this time (bp=162±5), and was significantly lower than the other three groups. The 6-OHDA treatment did not produce any differences in the highly variable activity counts for the 4 groups (SHR-VEH=205±80; 6-OHDA=217±87; 6-OHDA·DMI=205±34; for comparison the counts for untreated normotensive Wistar-Kyoto controls=49±13).

Catecholamine Levels as % Control

Group	N	NE BS	NE TELE	NE HT	DA TELE	DA HT
6-OHDA	4	11	3	16	30	55
6-OHDA·DMI	6	80	97	76	60	96
VB	5	--	6	18	70	64

The results of this experiment suggest that while no clear pattern of CA depletion is associated with attenuation of the hypertension in SHR's, the 6-OHDA treatment does not affect this disease process in a nonspecific manner. In addition, the activity data suggest that the hyperactivity of SHR's is not a consequence of increased blood pressure. Further lesion studies are being conducted in order to replicate these preliminary results and to determine if lesions which reduce activity also affect blood pressure. (Supported by NIH Grants HL 19226 and NS 15600).

119.11 THE EFFECT OF INTRAVENTRICULARLY INJECTED DOPAMINE (DA) ON THE BLOOD PRESSURE OF THE CONSCIOUS RAT: EFFECT OF DICHLORONITROPHENOL (DCNP) AN INHIBITOR OF SULFOCONJUGATION. N.T. Buu*, J. Duhaime* and O. Kuchel*, (SPON: A. Barbeau), Lab. of Autonomic Nervous System, Clinical Research Institute of Montreal, Montreal, Quebec H2W 1R7, Canada.

Central dopaminergic pathways have been suggested to be involved in cardiovascular regulation. However reports on the effect of centrally applied DA on the blood pressure (BP) have been conflicting. Several workers found that DA elicited a pressor effect on the mean arterial BP while others reported a depressor effect or both effects depending on the animal species and whether the animals were conscious or under anaesthesia. DA on the other hand has been shown to readily undergo sulfoconjugation and recently we found that intraventricularly (IVT) injected DA sulfate produced a pressor response in rat. In the following study, we examined the cardiovascular response to various doses of free DA in the conscious rat, and using DCNP, an inhibitor of the sulfoconjugating enzyme for DA, we investigated the possibility that the pressor effect of DA may be due to its conversion to DA sulfate.

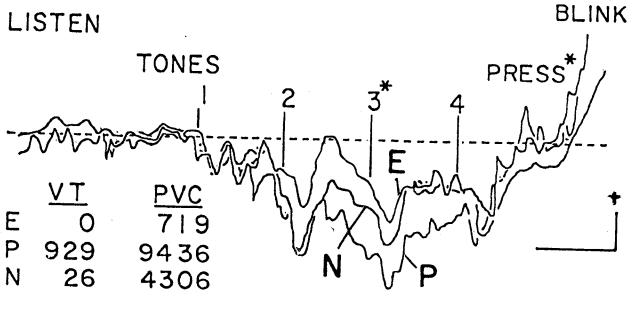
Male Sprague Dawley rats weighing approximately 200 g were injected IVT with free DA (5 µg-80 µg) and their BP were measured through a carotid artery catheter. Rats which showed a biphasic response to DA were divided into 2 groups. The first group was pretreated with DCNP and the second with the vehicle. Both groups were reinjected with DA and their BP was again monitored. Some rats had their cerebrospinal fluid (CSF) taken 5 minutes following the injection of DA for analysis of CA sulfates by a radioenzymatic method.

We found that at low doses (10-50 µg) DA had a depressor effect on the BP while at high doses (60 µg or higher) DA showed a biphasic effect: an increase in BP followed by a decrease. Only the pressor response was abolished by the pretreatment of rats with DCNP suggesting that DA sulfate may be responsible for the pressor response. Anaesthesia, which had been previously shown to suppress the pressor response to DA sulfate, also abolished the pressor response to DA without affecting its secondary depressor effect. Moreover, an increase in DA sulfate was detected in the CSF of rats treated with high doses of DA. Both effects of DA can be reduced or suppressed by haloperidol, metoclopramide, and hexamethonium indicating that central dopaminergic receptors were involved.

In summary the results of this study suggest that: 1) intraventricularly injected DA can produce both a pressor and a depressor effect on the BP of the rat; 2) the depressor effect is caused by free DA; 3) the pressor effect of DA may be due to its conversion to DA sulfate. (Supported by grants from the MRC).

119.12 THE EFFICACY OF ANTIARRHYTHMIC DRUGS IN CARDIAC PATIENTS IS PROPORTIONAL TO THEIR ABILITY TO REDUCE THE AMPLITUDE OF THE CEREBRAL EVENT-RELATED SLOW POTENTIAL. James E. Skinner, Marie-F. Montaron* and Craig M. Pratt*. Depts. Neurol. and Med., Sects. Neurophysiol. and Cardiol. Baylor College of Med., Houston, TX 77030

Previous studies in animals have shown that psychologic stress increases the vulnerability of the heart to arrhythmias (Skinner et al., Circulation, 1975). Stressful stimuli evoke event-related slow potentials (ERSPs) in certain regions of the brain (Skinner & Yingling, Electroencephalogr. Clin. Neurophysiol., 1976). The electrogenesis of the ERSP is related to catecholaminergic activity in the underlying tissue (Skinner et al., J. Neurochem., 1978). Neural blockade of the output pathway of the reactive cerebral system to the brainstem cardioregulatory centers prevents the increase in cardiac vulnerability evoked by stressful stimuli Skinner & Reed, Am. J. Physiol., 1981). Now we report that pharmacologic intervention by centrally acting drugs markedly reduces ventricular arrhythmias and diminishes the amplitude of the ERSP in patients with ischemic heart disease. A double-blind randomized treatment protocol was used in 4 subjects. The computer-averaged ERSP was recorded from the vertex of the scalp. It was elicited by asking the subject to listen to a series of 4 tone bursts and to press a button if the 3rd tone was missing (missing during 10% of bursts). Pharmacologic intervention by either Ethmozin (E), a phenothiazine, or Norpace (N), a class I antiarrhythmic drug, reduced total 24-hr premature ventricular contractions (PVC) and episodes of ventricular tachycardia (VT) compared to placebo (P) controls. The antiarrhythmic effect was proportional to the ability of the drug to reduce the amplitude of the ERSP. Norpace has a soporific side-effect, whereas Ethmozin could not be discriminated from placebo. We conclude that the antiarrhythmic effects of the drugs may result from their actions on the central nervous system.



119.13 EVIDENCE THAT THE CHOLINERGIC NEURONS OF THE BASAL FOREBRAIN CONTRIBUTE TO THE CORTICAL VASODILATION ELICITED BY FASTIGIAL NUCLEUS STIMULATION IN RAT. C. Iadecola, S. Mraovitch, M.P. Meeley and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Electrical stimulation of the rostral cerebellar fastigial nucleus (FN) in rat, elicits a powerful and global increase in cerebral blood flow (CBF). In the cerebral cortex the increase in CBF up to 240% of control is not secondary to increased metabolism nor to excitation of peripheral nerves innervating the cerebral vessels (Nakai et al., Brain Res. 1982, in press). We sought to determine: (a) whether the increase in cortical CBF is cholinergically mediated and, if so, (b) whether cholinergic neurons in basal forebrain (BF) which innervate the cerebral cortex (Proc. Natl. Acad. Sci. USA, 10, 5392, 1979) contribute to the cholinergic vasodilation. Rats were anesthetized (chloralose), paralyzed (tubocurarine) and artificially ventilated. At all times arterial pressure (AP) was maintained in the autoregulated range ($<$ 140 mm Hg) and blood gases were controlled. The FN was electrically stimulated (50 Hz; 1 sec on/1 sec off at 75-100 uA). CBF was measured in 13 brain areas by the ^{14}C-iodoantipyrine technique by regional dissection. As before, FN stimulation in 6 rats increased CBF in all areas, primarily in the cerebral cortex. The global increase in CBF was completely abolished in 5 rats by administration of atropine sulfate (0.3 mg/kg i.v.) 15 min before FN stimulation. To assess the contribution of the cholinergic neurons of the BF to the cortical vasodilation, unilateral electrolytic lesions were placed in the lateral preoptic area at the level of anterior commissure to interrupt the ipsilateral projection of cholinergic fibers to the cerebral cortex (Wenk et al., Brain Res. Rev. 2, 295, 1980). Two days after placement, regional CBF was measured. In 4 unstimulated controls, BF lesions alone produced a small (12-19%) but significant ($p < 0.025$) reduction in CBF, restricted to the ipsilateral cerebral cortex . In 5 rats the BF lesion greatly reduced the increase in cortical CBF associated with FN stimulation ipsilateral to the lesion. The maximal reduction (by 75%) was in frontal cortex (intact side: control 97 $\pm$ 5, FN stimulation 217 $\pm$ 27 ml/100gxmin; lesioned side: control 85 $\pm$ 4, FN stimulation 112 $\pm$ 9 ml/100gxmin); the least (by 23%) was in occipital cortex. In contrast, BF lesions did not change the increases in CBF elsewhere in brain. In 9 rats identical lesions of BF reduced choline acetyltransferase (CAT) activity ipsilaterally in cerebral cortex: frontal to -47%, parietal to -22% and occipital cortex to -11% of control. We conclude that: a) the global increase in CBF elicited by FN stimulation is mediated by muscarinic receptors in brain and/or on cerebral vessels and b) in the cerebral cortex the increase in CBF depends upon pathways originating in or passing through the BF. The results are compatible with the hypothesis that cholinergic neurons of the BF participate in the cortical cerebrovascular vasodilation elicited by FN stimulation.

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119.14 ULTRASTRUCTURAL EVIDENCE THAT VAGAL AFFERENTS TERMINATE ON CATECHOLAMINERGIC NEURONS IN THE NUCLEUS TRACTUS SOLITARIUS. K.K. Sumal, W.W. Blessing, T.H. Joh, D.J. Reis and V.M. Pickel. Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

We sought to determine whether primary sensory afferents of the vagus directly terminate on catecholaminergic neurons of the A2 region in the medial nucleus tractus solitarius (NTS). This was accomplished by combining radioautographic localization of ^{3}H-amino acids anterogradely transported from the nodose ganglia with immunocytochemical localization of tyrosine hydroxylase (TH). One ul (10 uCi) of L-proline (2,3,4,5,-^{3}H) and L-leucine (3,4,5-^{3}H) was injected unilaterally into the nodose ganglia of adult rats. Three days after nodose injection, the brains were fixed by aortic arch perfusion with 4% paraformaldehyde and 0.2% glutaraldehyde, coronally sectioned, immunocytochemically labeled for TH, and processed for light and electron microscopic radioautography. By light microscopy, reduced silver grains were heavily distributed throughout the rostrocaudal extent of the NTS. Radioautographic labeling was most extensive in the NTS ipsilateral to the nodose injection. Immunocytochemical localization of TH in perikarya and processes was equally evident in both solitary nuclei. However, superimposition of silver grains over TH-labeled processes was most evident ipsilateral to the nodose injection. By electron microscopy, reduced silver grains were exclusively localized to large (2-3 um) axon terminals containing numerous small, clear and a few large, dense, core vesicles. The radioautographically labeled terminals formed predominately asymmetric synapses with dendrites in the NTS. While many of the recipient dendrites were unlabeled, a substantial number exhibited immunoreactivity of TH. In addition to axodendritic synapses, the primary afferents occasionally formed junctions with TH labeled processes having the characteristics of axon terminals. These studies demonstrate that vagal primary afferents make monosynaptic synapses with both axons and dendrites of catecholaminergic A2 neurons in the NTS.

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120.1 CATECHOLAMINE REGULATION IN THE SYMPATHETIC NERVOUS SYSTEM OF THE SPONTANEOUSLY HYPERTENSIVE RAT, Noreen Tuross*, Vic Narurkar* and Robert L. Patrick. (SPON: Cecilia Giambalvo). Neuroscience Section, Division of Biology and Medicine, Brown University, Providence, RI 02912.

The present studies were undertaken in order to compare catecholamine regulation in the spontaneously hypertensive rat (SHR) and the normotensive (WKY) control strain. We were interested in testing two specific hypotheses: (1) that the sympathetic nervous system may be functioning at a higher rate of activity in the SHR and (2) that the sympathetic response to a stressful environment may be greater in the SHR compared to the WKY. Either of these conditions would support the concept that altered catecholamine functioning may be related to the development and/or maintenance of the hypertensive state.

Since conditions which increase sympathetic discharge in the rat, such as insulin-induced hypoglycemia, also produce an increase in soluble adrenal tyrosine hydroxylase activity via a neuronally-mediated mechanism (Patrick and Kirshner, Mol. Pharmacol. (1971), 7: 87-96), we have reasoned that if hypothesis number one is correct, we should observe an increase in adrenal tyrosine hydroxylase activity in the SHR compared to the WKY. As early as 5 weeks of age, however, and continuing up to at least 13 weeks of age, the adrenal tyrosine hydroxylase activity (assayed at a saturating 6-methyltetrahydropterine cofactor concentration) is significantly lower in the SHR compared to the WKY (e.g. 25.4 ±1.6 nmol dopa produced/hr/gland in the WKY vs. 14.9 ± 0.80 units in the SHR at 11-13 weeks of age, a statistically significant difference at a P value of less than 0.001).

In order to test the second hypothesis, rats were exposed to a 4°C environment for 24 hr. Adrenal tyrosine hydroxylase activity increased by 27% in the WKY, but showed a significantly greater increase (77%) in the SHR. Taken together, these data suggest (1) that the SHR may have a decreased sympathetic input to the adrenal gland under basal conditions, possibly in response to the hypertensive state, and (2) that under stressful conditions the adrenal tyrosine hydroxylase response may be greater in the SHR, as a result of either a greater sympathetic and/or greater cholinergic receptor response.

(Supported by the Rhode Island Chapter of the American Heart Association and by the Rhode Island Foundation)

120.2 IN VIVO ELECTROCHEMICAL DETECTION OF ALTERED ENDOGENOUS CATECHOLAMINE AND INDOLEAMINE RELEASE FROM RAT DORSAL RAPHE NUCLEUS IN RESPONSE TO PHENYLEPHRINE HYPERTENSION. Hirotoshi Echizen* and Curt R. Freed (SPON: Randall N. Pittman) Depts. Med. and Pharm. U. Colo. Sch. Med., Denver, CO 80262

Although numerous studies have revealed that the central monoaminergic neuronal systems play an important role in blood pressure (BP) regulation, few studies have attempted to measure endogenous neurotransmitter release in response to peripheral BP change. Since the dorsal raphe nucleus (DRN) has both serotonergic and catecholaminergic components and may have a role in BP regulation , we decided to study transmitter release in this region produced by phenylephrine(PE)-induced hypertension. Urethane anesthetized male Sprague-Dawley rats, 300-400gm, had femoral arterial and venous catheters inserted for BP monitoring and drug infusion, respectively. Neurotransmitter release was measured by in vivo electrochemistry with carbon paste electrodes stereotaxically implanted in DRN. Stainless steel and Ag/AgCl electrodes were also placed in brain. Electrochemical recording was done with a Bioanalytical System DCV-5 cyclic voltammetry apparatus with semiderivative signal processing. Electrodes were scanned at 10 mV/sec from -0.2 V to +0.5 V every 5 min during the experiment. Peaks at +0.15 V and +0.26 V were defined as the catechol and indole, respectively, according to the oxidation potentials observed in in vitro experiments. Peak identification was also evaluated by pharmacological experiments in vivo using alpha-methyl-p-tyrosine, p-chloroamphetamine, and pargyline. After basal catechol and indole release were measured for 30 min, PE infusion was begun to elevate BP 50 mmHg above baseline for 50 min. Drug infusion was then discontinued, but BP and transmitter release were measured for an additional 2 hr. Results show that indole release increased immediately after the initiation of hypertension, and remained elevated. By contrast, catechol release initially decreased during hypertension, but then increased and remained elevated in parallel with the indole response. The magnitude of change from basal values in transmitter release are shown below.

	⊢—PE Infusion—⊣				
Time (min)	0	25	50	100	150
Electrochemical signal ratio (mean±S.E.M.)					
Indole	1.00±.01	1.16±.08	1.67±.31*	2.16±.55**	1.96±.56**
Catechol	1.02±.02	0.84±.05	1.07±.09	1.35±.10*	1.20±.11

* $p<.05$, ** $p<.01$ by ANOVA; n=6 at each time point.

These data indicate that PE-induced hypertension produces rapid increase in indole release but an apparent reduction then increase in catechol release from dorsal raphe nucleus.

120.3 CHRONIC ARTERIAL HYPERTENSION FOLLOWING INJECTION OF SKF 91488 INTO THE HYPOTHALAMUS OF THE RAT, P.J. GATTI* and S.B. GERTNER* (SPON: R.D. HOWLAND), Dept. Pharmacol., UMDNJ-NJ Med. Sch., Newark, N.J. 07103.

SKF 91488 or S-[4-(N,N-dimethylamino)-butyl]isothiourea is a potent inhibitor of the enzyme histamine-N-methyltransferase, which has no histamine agonist activity per se. When injected intracerebroventricularly (i.c.v.) in the conscious rat, it produces an acute rise in mean arterial pressure (MAP) and heart rate (HR), and these actions can be blocked with simultaneous use of H-1 and H-2 receptor blockers. Thus, the compound acts by increasing endogenous brain histamine. The site(s) for the action of SKF 91488 within the brain when given i.c.v. are unknown. It was for this reason that we injected the drug into the anterior hypothalamic area (AHA) and the posterior hypothalamic nucleus (PHN), sites where histamine is believed to produce its cardiovascular actions. Injection of 5-10 µg/0.5 µl of the compound produced no immediate effect on MAP or HR when injected unilaterally into these areas in conscious freely moving rats. However, 24 hours after these injections were made, and 10 µg had been injected into the AHA, an increased MAP of 33 ± 2.8 mmHg (n=6) was observed, when compared to predrug values ($p<0.001$). The day following injection of this compound, we observed greater fluctuations in blood pressure than were seen the previous day. In addition, an increase in HR was evident concomitant with the changes in MAP (87 ± 24.9 bpm, $p=0.025$). This increase in HR varied greatly ranging from 40-180 bpm and was not related to predrug values. Core body temperatures were consistently elevated to 39-40°C in these animals. Injection of SKF 91488 into the PHN produced similar responses. When injections were made both in the AHA and the PHN in the same animal, the cardiovascular responses seen were even greater than those observed after injection into either area alone. Many of the animals showed changed behavioral patterns after the injections such as increased aggressive behavior and increased reactivity to external stimuli. Injection of the same volume of artificial cerebrospinal fluid into these areas had no effect on MAP, HR or body temperature. Histological examination of the brains of the treated animals revealed lesions of those areas injected with SKF 91488. It is quite possible that these lesions are responsible for the physiological effects either by eliminating an inhibitory center or stimulating an excitatory center of cardiovascular control in the central nervous system. The mechanism for production of the lesions is unknown.

120.4 CORTICOTROPIN-RELEASING FACTOR: MECHANISM TO ELEVATE MEAN ARTERIAL PRESSURE AND HEART RATE. Laurel A. Fisher* and Marvin R. Brown. Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Various stressful stimuli evoke concurrent activation of the pituitary-adrenal axis and the sympathetic nervous system. The hypothalamic hormone, corticotropin-releasing factor (CRF), initiates the corticoid response to stress by stimulating pituitary ACTH release. This peptide also acts within the brain to raise circulating catecholamine (CA) levels, elevate oxygen consumption, produce hyperglycemia and increase mean arterial pressure (MAP) and heart rate (HR). Thus, CRF may be a possible mediator of the integrated endocrine and autonomic responses to stress. The following studies were undertaken to investigate the mechanism by which CRF acts within the brain to influence cardiovascular function.

Male Sprague-Dawley rats (200-250 g) bearing chronic indwelling jugular venous, femoral arterial and lateral ventricular cannulae were used in all experiments. Intracerebroventricular (icv) administration of CRF (20 pmoles, 200 pmoles, 2 nmoles) produced dose-related increases in MAP and caused moderate tachycardia. These cardiovascular changes were rapid in onset (by 5 min) and persisted throughout the 60 min time course. Pretreatment with the ganglionic blocker, chlorisondamine (3 mg/kg, ip), abolished both the increased plasma CA levels and the elevated MAP and HR normally observed after CRF treatment. However, CRF increased MAP and HR in adrenalectomized rats and in rats given ODT8-SS (1 nmole icv) suggesting that CRF-induced cardiovascular changes are not dependent on adrenal CA release. Neither hypophysectomy nor dexamethasone pretreatment prevented CRF-induced increases in MAP and HR, suggesting that the cardiovascular actions of CRF are not mediated via pituitary ACTH or β-endorphin release. CRF also elicited increased MAP and HR in rats treated with captopril (2 mg/kg, iv) indicating that CRF-induced cardiovascular changes do not depend on the peripheral renin-angiotensin system. In contrast to our findings in dogs, icv injections of CRF do not elevate plasma vasopressin levels in rats so it is unlikely that the central pressor actions of CRF involve vasopressin secretion.

These results demonstrate that CRF acts within the brain to increase MAP and HR by stimulating sympathetic nervous outflow. Whereas CRF elevates circulating levels of both epinephrine and norepinephrine, the above studies suggest that enhanced norepinephrine release mediates the cardiovascular changes elicited by icv administration of CRF. Experiments are currently underway to determine the effects of CRF on norepinephrine turnover in various peripheral tissues.

120.5 INFLUENCE OF DIETARY SODIUM ON NORADRENERGIC TRANSMISSION IN HYPERTENSIVE RATS. M.J. Meldrum, L. Badino* and T.C. Westfall. Department of Pharmacology, St. Louis Univ. School of Medicine, St. Louis, MO 63104.

Dietary sodium has been implicated as a factor in the pathogenesis of hypertension in several animal models, and a neurogenic component involving increased noradrenergic nerve activity has also been proposed in these models of hypertension. We have previously studied the effects of dietary sodium on adrenergic neurotransmission in normal wistar rats. No differences in ^{3}H-NE release or maximal tension development in portal veins of rats on normal or low sodium diets was observed, while there was an enhancement of stimulation induced release of ^{3}H-NE in the A_2 region of the NTS (Fed. Proc. 41:8040, 1982). The present study extends these observations on the effects of dietary sodium to hypertensive animals. Stimulation induced ^{3}H-NE release was measured in portal veins, hypothalamus and A_2 region of NTS in three age groups of WKY and SHR (5-6 week prehypertensive; 10-11 week young hypertensive; and 28 week mature hypertensive animals). The portal vein was incubated with ^{3}H-NE for 30 min, followed by an 80 min washout period. Tissues were electrically field stimulated at increasing frequencies (1,2,5,10 Hz) for a total of 480 pulses at 15 min intervals. The stimulation induced release of ^{3}H-NE was less from vessels obtained from both SHR and WKY maintained on a low sodium diet, compared to a normal diet. In addition, release from the portal vein of the SHR was lower than that of the WKY at 6 and 11 weeks of age. The hypothalamus and the A_2 region of the NTS were removed, incubated with ^{3}H-NE for 30 min, placed in superfusion chambers, field-stimulated, and the overflow of ^{3}H-NE measured. In the hypothalamus, stimulation induced ^{3}H-NE release decreased with development of hypertension in the SHR, while release in the WKY animals remained constant on the normal sodium diet. The release of ^{3}H-NE was higher at 5-6 weeks, equal at 10-11 and lower at 28 weeks in SHR compared to age matched WKY animals. In animals on low sodium diets, release of ^{3}H-NE was decreased in both WKY and SHR at all ages. In the A_2 area the release from SHR remained constant while that in the WKY increased with age, suggesting an increase in the inhibitory output of the A_2 area on sympathetic outflow from the CNS in the WKY animals but not in SHR. The low sodium diet resulted in no changes in release of ^{3}H-NE from the A_2 area. These results suggest that lowered dietary sodium may result in specific changes in stimulation induced ^{3}H-NE release from portal vein and hypothalamus. The stimulation induced release in hypothalamus and A_2 region also showed differences which occur with hypertension development.

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120.6 THE CONTRIBUTION OF THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) REGION IN HYPERTONIC SODIUM CHLORIDE INDUCED HYPERTENSION. J.R. Haywood*, N.W. Ball*, M.D. Lifschitz*, T. Brennan* (SPON: T.M. Mikiten). Depts. Pharmacology and Medicine, Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

The AV3V region is an osmosensitive site necessary for the production of pressor responses to intracerebroventricular hypertonic stimuli. Activation of osmoreceptors by the intravenous administration of hypertonic NaCl to bilaterally nephrectomized animals has recently been shown to increase arterial pressure (AP) by the stimulation of vasopressin (AVP) release Hatzinkalaou, et al. AJP 240:H827, 1981). The purpose of this study was to assess the role of the AV3V in the increase of AP caused by the peripheral administration of hypertonic saline. AV3V lesioned or sham lesioned rats were prepared with femoral artery and vein catheters and bilaterally nephrectomized under methoxyflurane anesthesia. At least 3 hrs later, each animal was infused with 10 meq/kg sodium (Na^+) at a rate of .0103 ml/min. During the infusion, plasma osmolality increased 33.8±0.9 mOsm/kg. in the sham lesioned rats and 43.2±2.9 mOsm/kg. in the AV3V lesioned animals. AP rose from 131.0±2.8 mmHg to 166.5±1.8 mmHg in the sham lesioned rats within two hours. Heart rate (HR) decreased from 408±15 bpm to 315±13 bpm. In the lesioned rats, AP increased from 107.2±3.8 Hg to 121.6±4.0 mmHg during the two hour infusion period. In these rats, HR decreased from 425±18 bpm to 385±20 bpm. To assess the contribution of the sympathetic nervous system (SNS) to the elevation in AP in both groups of rats, atropine (0.2 mg/kg) and hexamethonium (25 mg/kg) were administered to achieve total ganglionic blockade. Ten minutes later, the vasopressin antagonist d$(CH_2)_5$VDAVP, (10 µg/kg) was given to determine the role of vasopressin (AVP). Total ganglionic blockade reduced AP by -24.6±4.6 mmHg in the sham lesion animals and by -34.8±1.5 mmHg in the AV3V lesion rats, indicating a greater contribution of the SNS in lesioned rats. Heart rate increased to 324±9 bpm in the sham lesioned rats and 332±19 bpm in the AV3V lesioned animals. The addition of the AVP antagonist caused a further reduction in pressure of -74.4±4.4 mmHg in the sham lesion rats and -30.6±4.3 mmHg in the AV3V lesion animals, suggesting a greater release of AVP in the sham lesion rats. Heart rate did not significantly change in either group of rats in the presence of the AVP antagonist. Thus we have confirmed that this acute model of hyperosmotic induced hypertension is caused by vasopressin induced vasoconstriction. Furthermore, AV3V lesioned animals are protected against a rise in AP to peripheral hyperosmotic stimuli because of an apparent inability to release AVP. (Supported by AHA, Texas Affiliate and NIH HL 26993).

120.7 DIFFERENTIAL PATTERNS OF SYMPATHETIC OUTFLOW INITIATED BY CENTRAL EFFECTS OF ANGIOTENSIN AND SODIUM CHLORIDE. J. Tobey*, H. Fry*, C. Mizejewski*, G. Fink*, L. Weaver. Depts. of Physiol. and Pharmacol. & Toxicol., Mich. State Univ., E. Lansing, MI.

Angiotensin II (AII) and hyperosmotic stimuli such as hypertonic sodium chloride cause pressor responses which may be mediated in part by the sympathetic nervous system. These substances are known to act on central nervous system receptors; however, the inhibitory or excitatory nature of effects upon central sympathetic outflow are not well defined and uniformity of sympathetic responses is undetermined. This study was undertaken to further evaluate the potential contribution of the sympathetic nervous system to cardiovascular responses to systemic or intracerebroventricular administration of angiotensin or hypertonic sodium chloride. Experiments were performed in chloralose-anesthetized, vagotomized cats in which arterial baroreceptors had been denervated. Splenic, renal, and splanchnic sympathetic efferent nerve activity and systemic arterial pressure were recorded. Discharges of two nerves were recorded simultaneously to allow two sympathetic responses to be compared in individual animals. Stimulation was accomplished by administering 15 µg AII (0.3 ml) or 600 mM NaCl (3 ml) into the carotid artery or 1.0 µg (0.02 ml) AII or 600 mM NaCl (0.05 ml) into the left lateral cerebral ventricle. Injection of hypertonic NaCl into the carotid artery produced excitation of splenic nerve activity and significant inhibition of renal nerve activity. When hypertonic NaCl was injected into the cerebral ventricle, a slight excitatory response was elicited in the splenic nerve whereas, renal nerve activity was unchanged. Injection of AII into the carotid artery caused significantly greater splenic than renal excitation. Significant excitation of splenic nerve activity was produced by intracerebroventricular administration of AII whereas, renal nerve activity was unaffected by this stimulus. Preganglionic splanchnic nerve responses were similar in magnitude and duration to postganglionic splenic nerve responses to AII and hypertonic NaCl. These findings illustrate that stimulation of sodium-sensitive and angiotensin-sensitive receptors produces differential responses in sympathetic outflow. These responses appear to be initiated by actions of these substances on the central nervous system. These differential excitatory and inhibitory sympathetic responses may contribute to the complex cardiovascular responses to increased plasma concentrations of angiotensin or sodium. This research was supported by grants HL21436 and HL24111 from the National Heart Lung and Blood Institute.

120.8 DISSOCIATION OF CENTRAL ANGIOTENSIN II AND SODIUM PRESSOR SYSTEMS FROM MECHANISMS OF DEVELOPMENT OF 1K-RENAL AND STEROID/SALT HYPERTENSION IN THE RAT. Diane K. Hartle and Michael J. Brody. Dept. of Pharmacol. and the Cardiovascular Center, Univ of Iowa, Iowa City, IA 52242

A hypothalamic vasoconstrictor pathway is involved in the pressor responses produced by intracerebroventricular (icv) administration of angiotensin II (AII), hypertonic saline (HS) and carbachol (C). The pathway originates in the anteroventral third ventricle (AV3V) region, follows a periventricular route through anterior and ventromedial hypothalamic regions. Electrolytic lesions or knife cuts that transect the projection bilaterally eliminates the pressor responses to icv AII, HS and C, the central component of the pressor response to peripherally administered AII, and attenuates the development of renin-dependent hypertension (aortic ligation model). The present experiments were designed to determine if this vasoconstrictor system also contributes to pathogenesis of non-renin-dependent hypertension (1K Grollman and steroid/salt models). Rats with horizontal anterior hypothalamic knife cuts in the region rostral to the paraventricular nuclei but caudal to the AV3V region, underwent unilateral nephrectomy and wrapping of the contralateral kidney. Sham-operated animals served as controls. Blood pressures were monitored by tail cuff technique for 3 weeks. Control and knife cut groups had blood pressures that averaged 128 ± 6 and 125 ± 2 mmHg before renal wrap. Three weeks after wrap, blood pressures averaged 194 ± 3 and 195 ± 3 mmHg respectively. In another experiment, knife cut and control animals were administered 15 mg deoxycorticosterone acetate twice weekly and given 0.9% saline to drink. Blood pressures of the control and knife cut groups averaged 123 ± 3 and 120 ± 4 mmHg respectively before steroid/salt treatment. Twenty-four days after initiation of the treatment, these groups averaged 182 ± 4 and 179 ± 5 mmHg respectively. Histological analyses confirmed the bilateral transection of tissue critical to the vasoconstrictor pathway in each animal. These data indicate that the hypothalamic vasoconstrictor system for AII, HS and C is not necessary for development of 1K-Grollman or steroid/salt hypertension. Since electrolytic lesion in the AV3V region prevents both renin-dependent renal hypertension and renin-independent renal and steroid/salt hypertension, these data suggest that the AV3V region participates in these models of hypertension by separate mechanisms. In non-renin-dependent hypertension, the participation of a distinct vasoconstrictor projection, identified by electrical stimulation, and traversing from the AV3V region through the medial forebrain bundle (Fed. Proc. 41:1258, 1982) is currently under investigation. Supported by Grants HLB-14388 and HLB-07121.

120.9 BLOOD PRESSURE RESPONSES TO INTRACEREBROVENTRICULAR INFUSIONS OF ANGIOTENSIN II IN RATS WITH BORDERLINE HYPERTENSION. J. W. Hubbard*, J. E. Lawler*, G. F. Barker*, and L. Botticelli (SPON: J. F. Lubar). Psychophysiology Laboratory, Department of Psychology, University of Tennessee, Knoxville, TN 37916 and the Addiction Research Foundation, Palo Alto, CA.

The discovery of angiotensin II (AII) containing neurons within the central nervous system (Fuxe et al., Central Actions of Angiotensin and Related Hormones, 1977) and the isolation of AII receptors in the mammalian brain (Bennett and Snyder, J. Biol. Chem., 251:7423, 1976) have indicated that this peptide may play a role in the central regulation of blood pressure and fluid electrolyte balance. Consequently, abnormalities in neural responsiveness to central or systemic AII may play a role in the etiology and/or maintenance of certain forms of hypertension. Recent studies (McDonald et al., Endocrinol., 107:1305, 1980) indicate that the maintenance of elevated arterial blood pressure in the spontaneously hypertensive rat (SHR) may be mediated by the actions of AII on the central nervous system.

The purpose of the following pilot study was to examine the acute effects of intraventricular AII on blood pressure responses in F_1 (offspring of SHR x WKY) rats with borderline hypertension (systolic blood pressure (SBP)=150±10 mmHg) and normotensive WKY rats (SBP=116±8 mmHg).

Eight 12 week old male rats served as subjects (F_1, N=4; WKY, N=4) for this pilot study. Subjects were randomly assigned to one of two AII treatment groups, 5 ng/ul, or 500 ng/ul. All animals were anesthetized with xylazine (35 mg/kg, i.m.) followed by ketamine HCL (90 mg/kg, i.m.). The left femoral artery was then cannulated for direct measurement of arterial blood pressure. Following the arterial catheterization, the rat's head was mounted in a stereotaxic instrument (David Kopf).

Five minutes of baseline blood pressure and heart rate were recorded for each subject prior to the infusion of AII. The tip of a Hamilton 10 ul syringe was lowered into the third ventricle and 1 ul of AII was delivered over a three minute period.

Both F_1 and WKY animals showed significant increases in SBP to intraventricular AII.

	SBP (mmHg)		
	Baseline	50 ng	500 ng
F_1	104± 3	141±21	173±11
WKY	108±11	122±12	131± 9

The enhanced pressor response in F_1 animals may represent fundamental differences between F_1 and WKY rats with regard to AII receptor characteristics (affinity, sensitivity, or concentration), or effector mechanisms (sympathetic outflow or release of vasopressin).

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120.10 NEONATAL HYPERTHYROIDISM: EFFECTS ON SYMPATHETIC NERVOUS SYSTEM RESPONSES OF ADULT RATS TO ACUTE STRESS. R. McCarty, R. F. Kirby* and P. C. Brunjes. Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22901.

Thyroid hormones play an important role in regulating processes of cellular growth and nervous system development in mammalian neonates. In general, treatment of developing animals with triiodothyronine (T_3) or thyroxine (T_4) results in precocious cellular development and synaptogenesis but deficiencies in somatic and brain growth in adulthood. Recently, Lau and Slotkin (J. Pharmacol. Exp. Ther., 208:485, 1979) demonstrated that neonatal hyperthyroidism in rats was attended by an accelerated maturation of sympathetic and adrenal medullary responses to reflex activation of central sympathetic outflow. In the present study, we examined the effects of neonatal hyperthyroidism on the responsiveness of the sympathetic nervous system of adult animals to acute stress. Hyperthyroidism was produced in Long-Evans hooded rat pups by injections of T_4 (1 µg/g body weight) on postnatal days 1-4. Littermate controls received injections of the vehicle only. In adulthood, male rats (approximately 325 g) of the two groups were implanted with tail artery catheters to allow for repeated sampling of blood and direct measurement of mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min) in freely behaving animals. Two days after surgery, blood samples (0.5 ml) were collected and direct measures of MAP and HR were obtained while rats were resting in their home cages. Rats were then stressed by exposure to 1 minute of inescapable footshock (2.0 mA, 0.6 sec duration, every 6 sec). Additional blood samples and cardiovascular measures were obtained immediately and 5 minutes after termination of footshock. The activity of the sympathetic-adrenal medullary system was assessed by measuring plasma levels of norepinephrine (NE) and epinephrine (EPI) using a radioenzymatic assay. Basal values for plasma NE and EPI and MAP did not differ between T_4 and control rats. However, basal HR was elevated in T_4 rats. Footshock-induced increments in plasma levels of both catecholamines were significantly greater in T_4 compared to control rats even though behavioral responses to footshock were similar for rats of the two groups. Our findings indicate that neonatal treatment with T_4 results in a hyperresponsiveness of the sympathetic-adrenal medullary system to acute stress which persists into adulthood.

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120.11 BILATERAL LESION OF CENTRAL AMYGDALOID NUCLEUS ATTENUATES HEMODYNAMIC RESPONSES TO NOISE STRESS IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR). T.M. Galeno, G.W. Van Hoesen and M.J. Brody. Dept. Pharmacol., Depts. Anatomy and Neurology, and the Cardiovascular Ctr., Univ. of Iowa, Iowa City, Iowa 52242.

The spontaneously hypertensive rat (SHR) exhibits exaggerated cardiovascular responsiveness to environmental stimuli. Previous studies from this laboratory have shown that compared to Wistar Kyoto controls (WKY), SHR exhibit an increased arterial pressure response to noise stress, produced hemodynamically by a combination of tachycardia and enhanced mesenteric and renal vasoconstriction. We have also shown that the cardiovascular responses to noise stress are similar to those elicited by electrical stimulation of the central amygdaloid nucleus in conscious SHR. Recent experimental neuroanatomical studies demonstrate clearly that the central amygdaloid nucleus is in a pivotal position to link sensory information with brain stem and hypothalamic areas known to have a role in cardiovascular regulation. The purpose of this study, carried out in conscious rats, was to determine if bilateral lesion of the central amygdaloid nucleus might alter the exaggerated cardiovascular responsiveness of SHR to noise stress. Twelve to 24 week old rats received bilateral lesions or a sham operation and were instrumented for chronic blood pressure and flow recording. Miniature pulsed Doppler flow probes were placed on the superior mesenteric artery, the renal artery and the lower abdominal aorta. A femoral cannula was used for arterial pressure and heart rate measurement. Both groups were exposed to noise (110 Decibel for 30 sec.) and lesions were histologically verified. Rats which had bilateral lesions that encompassed the central amygdaloid nucleus or its output pathways demonstrated significantly attenuated arterial pressure, heart rate, and mesenteric and renal vascular responses to noise. Noise was accompanied by a similar decrease in hindquarter vascular resistance in both groups. These data suggest that the central amygdaloid nucleus and its output pathways mediate the exaggerated cardiovascular responsiveness to noise stress in SHR. Since we demonstrated earlier that amygdalectomy attenuates development of hypertension in SHR, the present studies provide direct support for the hypothesis that limbic system mediation of exaggerated cardiovascular responsiveness contributes to the pathogenesis of spontaneous hypertension. (Supported by USPHS Grants HLB-14388 and GM 07069).

120.12 CARDIOVASCULAR RESPONSES TO ELECTRICAL STIMULATION OF THE A5 REGION IN THE RABBIT. M. L. Woodruff and R. H. Baisden. Department of Anatomy, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, TN 37614

Loewy et al. (Brain Res., 178, 196, 1979) have reported blood pressure (BP) increases without alterations in heart rate (HR) as a consequence of electrical stimulation of the adrenergic A5 area of the rat pons. This observation was replicated in the rabbit in the present series of experiments. The relationship between stimulation of the A5 region and baroreceptor input was also examined. All experiments were performed in pentobarbital-anesthetized preparations. Arterial BP was recorded from the abdominal aorta via a catheter inserted into the femoral artery. In most preparations changes in HR were recorded with a cardiotachograph. Stimulation of the A5 region (concentric bipolar electrode;10-75µA;60 Hz;1-5 sec duration) increased arterial BP without altering HR. BP was similarly increased by stimulation of the A5 region in rabbits given ipsilateral hemisections of the midcollicular midbrain 3 weeks prior to the experiment. Stimulation of the pons dorsal to the fibers of the VIIth nerve produced little or no BP alteration in any of the preparations. Local injection of the neurotoxin 6-hydroxydopamine into A5 eliminated BP responses to stimulation at current levels below 100µA. Increases were produced at intensities above 100µA. In normal rabbits stimulation of the aortic depressor nerve (ADN) concurrently with A5 stimulation attentuated the A5-induced BP increases. Bilateral destruction of the nucleus and tractus solitarius (NTS) from about 1 mm rostral to the level of the obex caudally for about 2.5 mm caused an increase in resting BP and HR, eliminated ADN-induced BP decreases and bradycardia, but had no effect on BP increases induced by electrical stimulation of the A5 region.

These results agree with those of Loewy et al. and are interpreted to indicate that electrical stimulation of the A5 region produces BP increases mediated by noradrenergic axons. This effect is not mediated by ascending pathways (i.e. to hypothalamus), or by projections to the NTS, but by projections to the spinal cord. These experiments do not definitely indicate that the cell bodies of the A5 region are responsible for the stimulation-induced BP increases. Stimulation of axons of passage arising from more rostrally lying noradrenergic cell groups may be responsible for the observed increases in BP, as stimulation of the cell bodies of the A5 group with monosodium-L-glutamate has been reported to produce decreases in HR and BP (Neil and Loewy, Neuroscience Abstracts, 7, 631, 1981).

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120.13 SYMPATHETIC NERVES AND ADRENAL MEDULLA: DIFFERENTIAL CONTRIBUTION TO BLOOD PRESSURE AND HEART RATE RESPONSES ELICITED BY EMOTIONAL STIMULI. A. Sakaguchi, J.E. LeDoux and D.J. Reis, Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Spontaneously hypertensive rats (SHRs) exhibit a biphasic pressor response and tachycardia during the presentation of conditioned emotional stimuli (LeDoux et al., Hypertension, in press). In the present study, we have sought to determine whether the sympathetic nerves and adrenal medulla contribute differentially to these cardiovascular conditioned responses (CRs). SHRs were subjected to classical fear conditioning (30 trials) during which the unconditional stimulus (US), an electric footshock (0.5 sec, 1.5 mA), was delivered at the termination of the conditional stimulus (CS), a pure tone (82 db, 800 Hz). Following conditioning, cannulas were placed in a carotid artery and jugular vein. The adrenal medulla was also removed bilaterally in some SHRs. Mean arterial pressure (AP) and heart rate (HR) were recorded using computer-assisted techniques. Unoperated and adrenal demedullated (AMX) SHRs received (i.v.) either guanethedine sulfate (GU; 10 mg/ml/kg), to produce acute chemosympathectomy, or saline (1 ml/kg) 15 min prior to the assessment of resting AP and HR and AP and HR CRs. Resting AP (in mmHg) was lower and resting HR (in bpm) was higher in GU (AP, 101 ± 2, $p < .05$; HR, 401 ± 6, $p < .05$) and AMX (AP, 146 ± 5, $p < .05$; HR, 392 ± 11, $p < .05$) than in saline controls (AP, 163 ± 4; HR, 364 ± 7). The conditioned pressor response in controls was biphasic and reached the first peak at the 3rd sec (25 ± 2 mmHg) and the second peak at the 7th sec (20 ± 2 mmHg) of the CS. The pressor response was accompanied by a tachycardia that peaked at the 5th sec of the CS (13 ± 2) but also increased after the cessation of the CS, reaching peak levels at the 5th sec following the CS (13 ± 3 bpm). AMX did not affect the pressor response (21 ± 3, 3rd sec; 16 ± 3, 7th sec) or the tachycardia (11 ± 2, 5th sec) during the CS, but suppressed ($p < .05$) the post CS rise in HR (1 ± 2, 5th sec after CS termination). GU reduced ($p < .01$) the first component of the pressor response (6 ± 1, 3rd sec) but enhanced ($p < .05$) the magnitude (23 ± 3) and duration (peak response 2 sec following CS) of the second component. GU+AMX abolished both the AP (1 ± 1, 3rd sec; 2 ± 1, 7th sec) and HR (-3 ± 1, 5th sec) CRs. We conclude: a) conditioned emotional responses in SHRs are accompanied by co-activation of the sympathetic vasomotor nerves and adrenal medulla; b) sympathetic nerve excitation is primarily responsible for the first component while sympathetic nerve excitation and adrenomedullary secretion both contribute to the second component of the pressor response; c) the secretion of catecholamines from the adrenal medulla accounts for the tachycardia following termination of the emotional stimulus. Cardiovascular responses during fear thus reflect differentiated responses to activation of the sympathoadrenal system. (Supported by HL 18974 and Merck, Sharp, and Dohme.)

120.14 INDEPENDENCE OF LOCOMOTOR ACTIVITY AND BLOOD PRESSURE IN ADULT HYPERTENSIVE AND NORMOTENSIVE RATS. D.G. Atwater*, J.E. Gellis*, W.C. Low, E.D. Hendley and D. Whitehorn (Sponsor: F.W. Marcoux). Dept. of Physiology, Univ. of Vermont College of Medicine, Burlington, Vermont 05405.

The spontaneously hypertensive rat(SHR) displays locomotor hyperactivity compared with Wistar-Kyoto(WKY) or Sprague-Dawley (SD) normotensive rats. We asked whether the hyperactive behavior was a direct consequence of elevated blood pressure in the SHR. Antihypertensive drugs were used to lower blood pressure (BP) in SHR and unilateral renal artery constriction was used to raise BP in WKY and SD rats. Locomotor activity was measured in an automated activity cage for 15 minutes (between 900 and 1200 h), 10 days after treatment or operation. The results showed that locomotor activity was not affected by alterations in BP.

(1) Adult SHR (16 wks) were treated with Captopril (100mg/kg/day) for 10 days. Control and treatment groups were matched for BP prior to the treatment period (n=6 in each group). After 10 days control BP was 178±4mmHg; Captopril treated BP was significantly lower (154±7mmHg; $p < .025$). Locomotor activity was not different ($p > .1$) in the control (415±53) and captopril treated (508±33) groups. Similar results were observed using hydralazine (20mg/kg/day in drinking water). After 10 days control BP was 212±9mmHg and 130±6mmHg in hydralazine treated ($p < .001$, n=5 in each group). No difference was observed ($p > .1$) in locomotor activity between hydralazine treated and untreated rats. These results demonstrate that lowering BP significantly in adult SHR has no influence on locomotor activity levels.

(2) Adult SD rats were made hypertensive by renal artery clip. Controls were sham operated. Ten days after surgery BP in sham SD was 128±4(n=5) and significantly greater in renal clip SD (179±5mmHg, n=4, $p < .001$). Locomotor activity scores were not different in SD shams and SD hypertensives (241±65 and 253±29 respectively). Unoperated SD have lower activities (148±8, n=5) indicating an influence of the surgical procedure.

Sham operated WKY had BP of 119±5mmHg (n=4). Renal clip WKY had significantly higher BP (164±2mmHg, n=4, $p < .001$). Locomotor activity scores were not different in WKY shams and WKY hypertensives (177±10 and 185±62). Unoperated WKY in our colony at this age have activities less than 100, again indicating a surgical effect. These results demonstrate that raising BP in genetically normotensive rats, SD or WKY, does not alter locomotor activity levels.

We conclude that locomotor activity differences between SHR and normotensive rats are not a direct consequence of differences in the level of systolic blood pressure. (Supported by HL24110 and Vermont Heart Association Grant in Aid (DW), PHS 5429-19-18 to the Univ. of Vermont (EDH), and PHS HL06338-01 (WCL)).

120.15 REINTERPRETATION OF THE RELATIONSHIP OF SPONTANEOUS HYPERTENSION TO BEHAVIORAL REACTIVITY AND PREWEANING HEART RATE. D.C. Tucker and A.K. Johnson, Preventive Medicine, Washington University School of Medicine, St. Louis, MO 63110, Department of Psychology University of Iowa, Iowa City, Iowa 52242.

One advantage of studying strains of animals genetically selected to become hypertensive is the possibility of identifying behavioral or physiological factors which are associated with the hypertensive process. Rats of the Spontaneously Hypertensive Rat strain (SHR) have been repeatedly demonstrated to have higher levels of locomotor activity when tested in an open field (OF) than rats from the normotensive strain from which they were bred (WKY) (see Tucker & Johnson, Neurosci. & Biobeh. Rev., 1981, 5, 463-471). Studies in our laboratory have documented early abnormalities in control of cardiac functioning in neonatal SHR (Tucker & Johnson, in press). The relationship between adult blood pressure and both adult locomotor activity and cardiac rate prior to weaning were critically examined in the present experiment.

Pups from 8 SHR and 8 WKY litters were implanted with subcutaneous silver wire electrodes at 15 days of age. Recordings of EKG were made from freely moving pups at 16, 20, 24, and 28 days of age. On days 60-64, rats were placed in an open field apparatus for 3 minutes and locomotor activity (grid crosses and rearing) and defecation were monitored. At 70 days of age, blood pressure (BP) was measured via an indwelling femoral artery catheter which had been implanted the previous day. Basal systolic, diastolic, and mean arterial BPs were scored at the end of a 20 minute recording period at a time when the animal was quiet. Data were analyzed using cannonical correlation to examine the relationship (a) between developmental trends in preweaning heart rate (HR) and BP measurements in adulthood and (b) the pattern of adult OF behavior during 5 days of testing and subsequent BP.

Previously observed differences between SHR and WKY rats in both preweaning HR and adult OF behavior were replicated in this experiment. However, when the relationship between locomotor activity and adult BP was examined separately within each strain, no significant association was observed ($\chi^2_{12}=6.5$, $p > .10$ for SHR, $\chi^2_{12}=8.9$, $p > .10$ for WKY). These data argue against assuming that increased locomotor activity observed in SHR is linked to their hypertension, either through a causal relationship or a common genetic etiology. Preweaning HR was similarly unrelated to adult BP levels when each strain was examined separately.

These data emphasize the importance of confirming apparent relationships between hypertension and behavioral or early developmental patterns by examing covariation withing strains or using behavioral genetics research strategies.

(NIH HLB-14338, 1 RO1 H 12302)

120.16 THE DEVELOPMENT OF SWIMMING BEHAVIOR IN SPONTANEOUSLY HYPERTENSIVE AND WISTAR-KYOTO RATS. M. B. Lansing* and A. K. Johnson (SPON: V. F. Bishop). Department of Psychology, University of Iowa, Iowa City, IA 52242.

Previous research from our laboratory has shown that spontaneously hypertensive rats (SHR) and their normotensive control strain (Wistar-Kyoto or WKY) differ in the time of appearance of eye opening and that a greater sympathetic influence appears in cardiac control at an earlier age in the SHR. The purpose of this study was to determine the developmental course of swimming behavior for SHR and WKY rats.

Schapiro et al. (Science, 1970, 168, 147-150) described the following developmental milestones in the swimming behavior of normal newborn Sprague-Dawley rats: At 6 days, poorly coordinated flexor and extensor movements are seen. By day 7, the rats begin to show flexion and contralateral extension. By day 12, well developed sequential extension and flexion of the front legs are seen and the nose is held out of the water. At day 15 or 16, there is the reappearance of a more random and irregular pattern of foreleg movement. The activity of the forelegs ceases by day 22 with the forelegs held in continuous extension in the adult form.

The appearance of these milestones was recorded in SHR and WKY rats and clear differences were found between these two groups. The SHR shows the lack of coordination in its flexor and extensor movements at day 6 and this activity continues until days 9-12. In contrast, the WKY exhibits well developed flexion and contralateral extension of the forelimbs at day 6. The behavior is coordinated until days 8-13 at which time the rat has difficulty maintaining its balance and corrects its position using its forelegs. Sequential extension and flexion of the forelegs reoccurs at day 13 for the WKY and begins to appear at days 9-12 in the SHR, and is well developed by days 14-22. The nose is out of the water by day 15 in the SHR and by day 12 in the WKY. The loss of coordination in the flexor and extensor movements reappears on days 23-28 in the SHR and on days 17-24 for the WKY rat. Complete cessation of foreleg activity occurs by days 24-30 in the SHR and on days 20-25 in the WKY.

Thus, there appears to be a dramatic difference in the pattern of the development of swimming behavior between the SHR and WKY rat. The behavior develops faster and follows a somewhat different developmental course in the WKY relative to the SHR. This study precedes others that will attempt to analyze hormonal and neural factors involved in the development of swimming behavior.

(Supported by NIH HLP-14338 and 1 RO1 H 12402.)

120.17 LESIONS OF PARAVENTRICULAR NUCLEUS REVERSE THE ELEVATED ARTERIAL PRESSURE AFTER AORTIC BARORECEPTOR DENERVATION IN THE RAT. T. X. Zhang* and J. Ciriello (SPON: M. A. Cook). Department of Physiology, The University of Western, Ontario, London, Canada N6A 5C1.

Aortic baroreceptor denervation in the rat is known to result in an elevation in arterial pressure which is associated with an increased norepinephrine turnover in the hypothalamus and brain stem. In addition, we have recently shown that renal denervation abolishes these effects on blood pressure and turnover and suggested that this was due to the removal of neural signals to the hypothalamus and brain stem which originate in the kidney. As the paraventricular nucleus of the hypothalamus (PVH) has been shown to play an important role in the regulation of the cardiovascular system and to contain neurons whose discharge rates are altered by electrical stimulation of aortic and renal afferent fibers, experiments were done to determine the role of this structure in the maintenance of the elevated arterial pressure after aortic baroreceptor (ADN) denervation in the rat. After recording arterial pressure (tail cuff method) and heart rate for a control period of one week the rats were randomly assigned to two groups. The first group was subjected to bilateral transection of the aortic and cervical sympathetic nerves and the second group to sham denervation. After one week, the first group was further randomly divided into a group in which small bilateral electrolytic lesions were made in the region of the PVH and a group that received sham lesions. Arterial pressure and heart rate were significantly higher in the ADN denervated group when compared to the sham denervated group (greater than 35 mmHg and 50 beats/min, respectively). Bilateral lesions of the PVH region significantly reduced arterial pressure and heart rate in the denervated animals compared to denervated-sham lesioned animals to levels which were not significantly different from pre-denervation values and from sham denervated-PVH lesioned animals. These data demonstrate that the PVH plays an important role in the maintenance of the increased arterial pressure and heart rate after ADN denervation and that these cardiovascular changes are dependent on the integrity of this structure. On the basis of previous evidence and the results of the present study it is suggested that the PVH is important site of integration of aortic and possibly renal afferent information in the regulation of circulation.

(Supported by the Ontario Heart Foundation)

121.1 MOTONEURON CONNECTIVITY IN EMBRYONIC CHICK LIMBS WITH AN ALTERED COMPLEMENT OF MUSCLE. C. Lance-Jones. Department of Anatomy and Cell Biology, SUNY-Downstate Medical Center, Brooklyn, N.Y. 11203.

Limb-innervating motoneurons form two longitudinal arrays within the lateral motor column of the spinal cord; a lateral group projecting to dorsally derived muscles and a medial group projecting to ventrally derived muscles. Following diverse experimental manipulations of the embryonic chick cord or whole limb, including early dorso-ventral limb reversals, this pattern is maintained. A possible source of cues enabling these two motoneuron classes to form correct connections is the developing target tissue itself. To examine this possibility, motoneuron connectivity is being assessed in chick embryos in which the complement of muscle tissue has been experimentally altered.

In one series of embryos, double dorsal (dD) hindlimbs were produced at Stage (St) 17-19 by replacing ventral limb bud tissue with a dorsal half limb bud from a donor embryo. At St 30-36 dorsal muscles and nerve trunks were present in duplicated form in the thigh. Ventral muscles were absent with the occasional exception of very proximal posterior ones. These results indicate that muscle regulation has not occurred and confirm earlier findings that gross nerve morphology is governed by limb structures. Both before (St 30) and after motoneuron cell death (St 36) motoneurons innervating thigh muscles in dD limbs were retrogradely labelled by horseradish peroxidase (HRP) injection into individual muscles. Host muscles (dorsal muscles in a dorsal position) were innervated by lateral motoneurons as in a normal embryo. However, donor muscles (dorsal muscles in a ventral position) were innervated by medial motoneurons in accord with their new as opposed to original position. The observation that motoneurons clearly sorted out into two classes despite the lack of two distinct muscle classes suggests that nerve sorting is separable from actual pathway choice and independent of target complement.

In a second experimental series, hindlimbs with only one set of dorsal muscles were produced. Ventral tissue removal at St 17-19 resulted in the absence of ventral musculature at St 36. HRP injections into selected dorsal muscles resulted in labelling of discrete clusters of lateral motoneurons as in a normal embryo. The motor column appeared depleted of motoneurons in medial regions where motoneurons innervating ventral muscles would normally be found. Examination of neuron outgrowth at earlier stages may suggest whether the two motoneuron classes behave independently or whether axon interactions, based on target type, occur.

Supported by NIH Grant NS1 7155-02 and the SUNY Research Foundation.

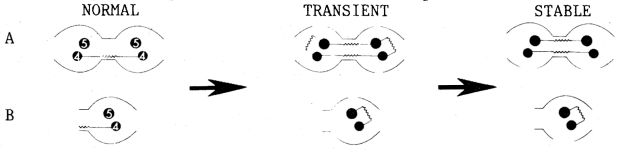
121.2 CHANGES IN CONNECTIVITY MAPS OF IDENTIFIED AXONS IN THE CRAYFISH DUE TO PARTIAL DENERVATION. William P. Hunt, Mary K. Worden* and Samuel J. Velez. Department of Biological Sciences, Dartmouth College, Hanover, N.H. 03755

The six axons that innervate the superficial flexor muscles of the crayfish distribute their innervation in a very specific pattern over the muscle surface (Velez & Wyman, J. Neurophysiol. 41: 75-96). When the nerve is cut the axons are capable of regenerating their connections with the same degree of specificity as observed in control animals (Ely & Velez, J. Neurophysiol. 47: 656-665). In previous work we have reported that different changes in the target area of the nerve affect the connectivity maps of some of these axons (Hunt & Velez, J. Neurophysiol. 47: 666-676; Hunt & Velez, Soc. Neurosci. Abstr. 6: 497, 1980; Clement, Hunt & Velez, Soc. Neurosci. Abstr. 7: 470, 1981). In all these experiments the neurons had lost all their connections and were thus regenerating a complete set of new terminals. Experiments are now being performed to study the effects of partial denervation of these neurons on their capacity to regenerate their connections. The nerve is cut at the mediolateral junction of the muscle, leaving the medial fibers with their normal complement of connections; the axons have to grow only into the lateral muscle field. This operation is going to affect these cells differently: axons 1 and 2 will lose very few terminals, axon 3 will lose half and axon 4 will lose most of its terminals. The animals were examined at regular intervals after the operation by monitoring the spontaneous activity of the nerve in isolated abdomens and matching the spike record with intracellular recordings of muscle junction potentials. Preliminary results indicate that all the axons are affected in the medial muscle population within one week after the operation. Their connectivity maps show a sharp decline in all medial regions which persist until the nerve begins to grow into the lateral fibers. After four weeks the lateral fibers become innervated and the medial and lateral connectivity maps of all the axons return to control values. This suggest to us that the intact terminals of these axons are temporarily affected by partial axotomy of the neuron.

(Supported by NIH Grant NS 13800 to SJV)

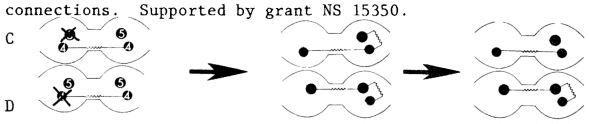
121.3 EXTANT SYNAPSES PLAY A ROLE IN THE SELECTIVE STABILIZATION OF NEW ELECTRICAL CONNECTIONS IN THE ADULT HELISOMA NERVOUS SYSTEM. A. G. M. Bulloch, H. R. Miller* and S. B. Kater. Dept. Zoology, Univ. of Iowa, Iowa City, IA 52242.

The selective formation of new transient and stable electrical synapses during neurite outgrowth in the adult nervous system of the snail Helisoma has been described previously (Science 212, 79-81; J. Neurophysiol., in press). We have hypothesized that the elimination of normally transient connections is contingent upon the development of a stable connection. This was based upon the observation that the connection between neurons 5 and 4 that is normally transient in paired buccal ganglia (A) is stable in single ganglia (B) in which the stable 5-5 synapse cannot develop. In preparing single ganglia, however, extant connections (e.g. 4-4), which could play a role in stabilizing new connections, are disrupted. For the present study we tested our hypothesis more rigorously by extirpation of individual neurons from paired ganglia before culture using a recently developed microdissection technique.



The normally transient 5-4 connection was examined during culture subsequent to removal of either one neuron 4, one neuron 5 or one neuron 19 (as a procedural control). As expected from previous work, 5-4 electrical coupling was established after short culture durations in all preparations, and was transient in control ganglia. After removal of one neuron 5, this interaction was also transient (C). In contrast, ganglia lacking one neuron 4 exhibited stable 5-4 coupling (D).

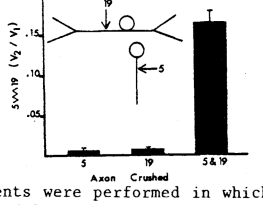
It is concluded that maintenance of an extant connection (4-4) is necessary for elimination of the new 5-4 synapse. Additionally, the development of the new, stable 5-5 connection is not sufficient to eliminate 5-4 coupling. Thus the signals responsible for eliminating usually transient synapses in this adult nervous system depend in part upon existing synaptic connections. Supported by grant NS 15350.



121.4 GROWING NEURITES ARE REQUIRED FOR FORMATION OF ELECTROTONIC CONNECTIONS IN MOLLUSCAN GANGLIA. R. D. Hadley and S. B. Kater. Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

Communication between many of the neurons in the buccal ganglia of the snail, *Helisoma* is via electrical synapses. Bulloch and Kater (*Science 212*: 79-81, 1982) found that axotomy-induced growth resulted in the formation of novel electrical connections. After esophageal nerve trunk (ET) crush and *in vivo* culture, buccal neurons fell into 2 general classes; those which always formed novel connections to neuron 5 and those which never did. Of the newly formed connections, one was stable (i.e. the 5-5 connection) and others were transient (e.g. 5-4). The present research is aimed at the basis for exclusion of some neurons (e.g. neuron 19) from the pool of neurons which were competent to form connections with neuron 5.

In isolated ganglia cultured *in vitro* for up to 1 week, results qualitatively similar to those *in vivo* were obtained. We observed stable 5-5 and transient 5-4 connections as well as absent or very weak 5-19 connections. Lucifer Yellow injections of the 3 neurons showed that neurons 5 and 4 produced extensive central sprouting after crushing ETs (which contain axons of 5 and 4, but not 19) while neuron 19 had very little or no central growth. This suggested a possible basis for neuron 19 exclusion from coupling to neuron 5: *perhaps only growing regions of neurons are capable of forming electrical connections.*



Experiments were performed in which selective nerve crushes axotomized either neuron 5 or neuron 19, or both neurons 5 and 19 (selectively evoking central growth). No 5-19 connections were observed with induced central growth only from neuron 19 or only from neuron 5. However, when both neurons 5 and 19 were induced to grow simultaneously, strong 5-19 connections ensued. Time course studies of 5-19 connections showed that these were transient, as were 5-4 connections.

These observations suggest the hypothesis that only growing neurites, as opposed to nongrowing regions of neurons, are competent to form electrotonic connections. Thus, 5-19 connections can occur only if both neurons are growing into a common environment. Supported by grant NS 15350.

121.5 SYNAPSE LOCALIZATION ON THE MAUTHNER CELL IS UNAFFECTED BY DISPLACEMENT OF A MAJOR AFFERENT PROJECTION. W.S. David and P.G. Model. Dept.Neurosci.,Albert Einstein Coll.Med.,Bx.,NY 10461

The spatial patterning of synapses in the vertebrate central nervous system is highly ordered. One theory suggests that spatio-temporal relationships between developing cell populations are responsible for this specificity. Another proposal attributes this ordering to the spatial restriction of discrete populations of axons to different regions of the brain, with connections forming where afferents and targets happen to meet (axonal segregation model). A third hypothesis suggests that cell surface markers underlie the organized spatial patterning of synapses (recognition model). We have disrupted the spatial relations between afferents and target cells in an amphibian embryo to test the spatio-temporal model. Mauthner cells (M-cells) are large identified neurons that occur as a single pair in the medulla of premetamorphic amphibians and fish precisely at the level of entry of the vestibular (nVIII) input. In the axolotl (Ambystoma mexicanum), the complete ipsilateral projection of nVIII to the M-cell is localized on the ventral surface and branches of its proximal lateral dendrite. nVIII terminals (club endings) are identifiable in both LM and EM. Prior to nVIII ingrowth, prospective ears and nVIII ganglia were unilaterally transplanted rostral or caudal to their normal site. The contralateral side served as a control. In the rostral series (1982, Anat.Rec. 202:41A), LM analysis of 21mm larvae revealed anatomically normal ears and brains. The hair cells of the transplanted ears were innervated by processes projecting out of a ganglionic complex of mixed V and VIII origin. This complex fed into the brain at the level of nV, rostral to the normal site of entry of nVIII. No portion of the nVIII ganglion was present at the level of the M-cell on the experimental side. EM analysis demonstrated club endings on the experimental M-cells with a distribution similar to that of the control cells. When ears were transplanted caudally, LM evaluation showed ears and brains of generally normal morphology. The transplanted ears were innervated, and nVIII ganglia appeared displaced and discrete (unmixed with other cranial ganglia). The experimental M-cells appeared relatively normal and preliminary EM analysis has revealed appropriately localized club endings.

Thus, in spite of the fact that the nVIII enters the brain at a foreign location and must course through unfamiliar territory, the experimentally introduced spatial distortions between afferents and target cells do not alter the pattern of synaptic connections on the latter. These data, together with those of Leber and Model (this volume) are consistent with a recognition or axon segregation model, but are inconsistent with a hypothesis that proposes a spatio-temporal mechanism as being sufficient to explain the ontogeny of highly ordered neuronal connections.
(Supported by NIH grants 5T32GM7288 and NS-07512)

121.6 NORMAL LOCALIZATION OF SYNAPSES ON THE AMPHIBIAN MAUTHNER CELL DESPITE PRECOCIOUS SYNAPTOGENESIS. S.M. Leber and P.G. Model. Dept. Neurosci., Albert Einstein Coll. Medicine, Bronx, NY 10461.

Afferents often form synapses on restricted regions of their target cells. The connections between vestibular (nVIII) axons and the Mauthner cell (M-cell) are an example of this sort of specificity. The M-cells are a uniquely identifiable pair of neurons in the brainstem of certain fish and amphibians. In the axolotl (Ambystoma mexicanum), the M-cell receives synapses from the ipsilateral nVIII only on the ventral surface and branches of the proximal portion of its lateral dendrite. The nVIII terminals (club endings) are distinctive at the EM level.

This specific distribution of terminals might result from nothing more than where and when during development the growing axons happen to meet the growing dendrites (spatio-temporal model). Alternatively, the distribution might reflect constraints on where the developing axons are capable of growing (axon segregation model (Kimmel, TINS 5: 47-50, 1982)) or synapsing (recognition model). To test the spatio-temporal model, we made nVIII axons form synapses on the M-cell before they normally would have and asked whether the eventual distribution of club endings was changed. Ears and vestibular ganglia from older animals (Harrison stage 33/34, just preceding axon formation) were unilaterally grafted in place of prospective ear/ganglion placodes of younger embryos (stage 23). LM and EM examination revealed that the axons had indeed precociously entered the brain and formed synapses on the M-cell. The M-cell's lateral dendrite was thus smaller and less mature than that which the axons normally reach.

We examined 21 mm feeding larvae to determine the localization of the club endings. The grafts had developed into anatomically normal ears and nVIII ganglia, with nerves entering the medullae at the correct location. The M-cells on the experimental side looked normal (except for one cell whose distal lateral dendrite bifurcated abnormally). Detailed EM mapping of club endings on the experimental cells revealed a distribution similar to that of the contralateral control cells.

Thus synaptogenesis initiated on a younger and smaller than normal M-cell does not alter the eventual localization of the synapses. We obtained the same result when we delayed the arrival of afferents by doing the reciprocal heterochronic transplantation (Soc. Neurosci. Abstr. 7, pg. 177, 1981). Together with the results of David and Model (this volume), these results conflict with the spatio-temporal model and require that the nVIII axons are either restricted to a certain region of the neuropil or are capable of recognizing a specific region of the M-cell surface.
Supported by NIH grants 5T32GM7288 and NS-07512

121.7 REGENERATION OF DEVELOPING SPINAL MOTONEURONS. Paul B. Farel. Dept. of Physiol., Univ. N. Carolina Sch. Med., Chapel Hill, NC 27514.

While axon elongation readily occurs following motor nerve transection, the specificity of innervation by motoneurons of their original target muscles is lost in both mammals and adult anuran amphibia. The present study was undertaken to determine whether developing spinal motoneurons similarly lack regenerative specificity. Experiments were performed on bullfrog tadpoles (Rana catesbeiana) because their large size permits surgical procedures to be performed with facility even at early stages of motoneuron differentiation.

The hindlimbs in frog are innervated by motoneurons of the lumbar lateral motor column (LMC). Muscles derived from the dorsal premuscle mass are innervated by motoneurons located ventrally in the LMC while muscles derived from the ventral premuscle mass are innervated by motoneurons located dorsally in the LMC. Further, distal hindlimb muscles tend to be innervated by motoneurons located more caudally in the LMC. The specificity of motor nerve regeneration thus can be determined by comparing the locations of motoneurons whose axons have regrown to a particular muscle with motoneuron locations found in normal animals.

Birth and migration of LMC motoneurons are completed during premetamorphic stages. Following application of the retrogradely transported histochemical marker horseradish peroxidase (HRP) to the ventral shank of premetamorphic tadpoles, 95% of the labeled motoneurons were located in the dorsal half of the LMC and were restricted to the caudal half of the lumbar enlargement. In experimental tadpoles, the three ventral roots contributing to the sciatic plexus were transected, and, following a 4-6 week interval, HRP was applied to the ventral shank. Retrogradely labeled motoneurons were now distributed throughout the LMC with no obvious tendency for localization along either the dorsal-ventral or rostral-caudal axes.

These results show that immature spinal motoneurons, at least at the developmental stages examined, have no markedly greater capacity for regeneration to their original target muscle than that of adult motoneurons.

121.8 TETRODOTOXIN BLOCKS THE FORMATION OF OCULAR DOMINANCE COLUMNS AND REFINED RETINOTOPOGRAPHY IN GOLDFISH. Ronald L. Meyer. Developmental and Cell Biology, Univ. Calif., Irvine, CA 92717.

To make ocular dominance columns, optic fibers that innervate posterior dorsal tectum were teased from surrounding "donor" tectum and inserted into a large mediolateral incision made at the anterior end of the opposite "host" tectum. This incision cut host optic fibers so that host and deflected optic fibers grew into host tectum at the same time and position. It was previously reported (Meyer, Neurosci. Abst., 7:405) that by 1 mo host and deflected fibers regenerated into dorsal posterior host tectum in overlapping fashion. By 2 mo, host and deflected fibers segregated into ocular dominance columns. To see if this process is sensitive to impulse blockade, activity was chronically blocked by biweekly injections of TTX into the vitreous. At various times after surgery, one eye was injected with proline for autoradiography. At 1 mo the innervation was indistinguishable from fish without TTX. At 2-3 mo, however, host and deflected fibers were evenly distributed in posterior dorsal tectum (no columns). Columns were also absent in a second group at 2-3 mo in which TTX injections were begun only at 1.5 mo postop. In a third group, TTX was given from 1.5 to 2.5 mo postop followed by 3 wks without TTX. Columns were present. In a fourth group, TTX was given throughout but only to one eye, host or donor. Columns were present at 2 mo. A fifth group was given subthreshold (nonblocking) TTX injections for 2 mo at which time columns were found.

A similar study examined the formation of retinotopography following simple optic nerve crush (no deflection). Retinotopography was assayed by making a small retinal lesion (sector mapping) or a half-retinal lesion (hemiretina mapping) immediately prior to proline labelling of eye for autoradiography. It was previously reported (Meyer, JCN, 189:273) that at 1-1.5 mo after crush, hemiretina mapping demonstrated gross topography while sector mapping revealed no refined topography (no denervated tectal sector). At 2-3 mo, sector mapping revealed refined topography (small denervated tectal sector). Fish were given TTX as above. At 1-1.5 mo or later, gross topography was always evident but no refined topography could be detected at 2-3 mo. In a second group TTX was begun 1.5 mo postop until sector mapping at 2.5 mo. No refined topography was detected. In a third group, TTX was given from 1.5 to 2.5 mo followed by 3 wks without TTX at which time sector mapping showed refined topography. A fourth group received a non-blocking dose of TTX and at 2.5 mo, sector mapping showed refined topography.

There is a late occurring, TTX sensitive phase in the formation of ocular dominance columns and refined topography.
(Supported by NIH grant NS 15381.)

121.9 ABNORMAL VISUAL INPUT DURING DEVELOPMENT LEADS TO ABNORMAL TRAJECTORIES OF ISTHMO-TECTAL AXONS IN XENOPUS FROGS. Susan B. Udin
Div. of Neurobiology, SUNY, Buffalo, NY 14214.

The Xenopus tectum receives visual input from both eyes. The contralateral eye's visual map is brought to the tectum by the optic nerve (retinotectal projection). The ipsilateral eye's map is brought to the tectum by an indirect pathway involving the nucleus isthmi (isthmo-tectal projection). These two maps normally are in register with each other. One of the developmental cues which serves to align the two maps is sensory input. For example, if early sensory experience is altered by rotating one eye, then isthmo-tectal topography changes in such a way as to bring the ipsilateral map into register with the contralateral map. (Udin and Keating, 1981, J.Comp.Neurol. 203:575-594).

How is this topography established? What paths do isthmo-tectal axons take en route to their final destinations? In order to trace the trajectories of individual isthmo-tectal axons, I have labeled axons by injecting horseradish peroxidase (HRP) into the nucleus isthmi. After three days, the opposite tectum is excised, flattened, fixed, and reacted using the heavy metal-DAB method (Adams, 1981, J.Histochem.Cytochem. 29:775). The pathways and arborizations of filled isthmo-tectal axons can be traced for their entire course within the tectum.

Most axons in normal tecta follow a fairly straight path between the point where they enter the tectum to the point where they arborize. Some branch as soon as they enter the tectum; these branches may diverge for a distance but then reconverge and terminate together.

In contrast, many isthmo-tectal axons in tecta of eye-rotated Xenopus have markedly abnormal routes. Some sweep in wide arcs before terminating. Some make sharp U-turns and switch-backs. Some appear to form terminals in more than one place. However, not all of the axons follow abnormal paths; some are indistinguishable from normal. At least some of these normal-appearing axons may have receptive fields corresponding to the center of the visual field of the rotated eye, since such fields would not be displaced by eye-rotation.

In conclusion, isthmo-tectal axons normally take fairly direct routes to their terminal sites in the tectum, but abnormal visual input can cause these axons to follow highly unusual pathways and to terminate in different locations from normal.

(This work was supported by NIH grant EY0347-01.)

121.10 CATECHOLAMINE DEPLETION DOES NOT PREVENT VISUAL SYSTEM PLASTICITY IN DEVELOPING XENOPUS FROGS. J.F.W. Deakin*, S.B.Udin, E.A. Dawes*, M.J. Keating* and S. Grant*. (Spon: J.S. Baizer).
Nat'l. Inst. for Med. Res., Mill Hill, London NW7 1AA, U.K. and Div. of Neurobiology, SUNY, Buffalo, NY 14214.

Kasamatsu & Pettigrew have reported that norepinephrine is necessary for monocular deprivation to alter ocular dominance in cat visual cortex (J.Comp.Neurol. (1979) 185:139-162). Is norepinephrine (NE) also required for visual system plasticity in developing Xenopus frogs?

In normal Xenopus, the contralateral retina sends a direct projection to the tectum and the ipsilateral eye sends an indirect projection to the tectum. These two maps are in register. If one eye is rotated during development, the ipsilateral projection compensates by changing orientation; this rearrangement brings the ipsilateral map back into register with the contralateral map. Visual input is necessary for this matching-up process (Keating, et al. (1975) Proc.R.Soc.Lond. 191:445-466).

To test whether NE innervation is also necessary, we injected 6-hydroxydopamine (6-OHDA) in ascorbate into the third ventricle of tadpoles at stage 56-63 of development and again four weeks later. In some animals, the right eye was rotated 45^{o}-135^{o} at the time of the first injection. When the animals were 5-20 weeks post-metamorphosis, we used electrophysiological mapping techniques to compare the orientations of the ipsilateral and contralateral projections of uninjected, ascorbate-injected and 6-OHDA injected frogs, some of which had a rotated eye. In frogs with both eyes in normal orientation, all maps were normal. In frogs with one eye rotated, we found a variety of results, as is typical for animals in the first few months after metamorphosis. Twelve of the ipsilateral maps were completely in register with the rotated contralateral maps ("all congruing"); eight ipsilateral maps had regions which did congrue and other regions where the topography was appropriate for a normal animal ("part congruing"); two ipsilateral maps showed no evidence of interaction with the rotated contralateral projection ("none congruing"). Following the recordings, we used high pressure liquid chromatography to analyze quantitatively the amounts of norepinephrine, epinephrine and dopamine in the brains between the level of the rostral medulla and rostral diencephalon (approximately 6-10 mg wet weight). Depletion of catecholamines ranged from 0%-98%. We found no relationship between completeness of depletion and degree of congruence of maps. Therefore, presence of NE does not appear to be necessary for visual plasticity in developing Xenopus.

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121.11 INTERNEURONAL COUPLING IN THE DEVELOPING RAT NEOCORTEX. L.S. Benardo, B.W. Connors and D.A. Prince. Department of Neurology, Stanford University School of Medicine, Stanford, CA 94305.

Electrical, and perhaps metabolic, coupling between cells is a prominent feature in the early development of most animal species. Since interneuronal coupling was recently described in adult neocortex of guinea pig, we wondered if coupling might play an important role in the ontogeny of neocortex. Slices of sensorimotor neocortex of albino rat pups were maintained in vitro for 8-10 hrs and standard intracellular techniques were employed. Lucifer Yellow CH was successfully injected intraneuronally (n=163) at varying subpial depths. Dye-coupling (i.e. the staining of more than one cell from a single cell injection) occurred in 70% of the injections recovered from cortex 1-4 postnatal days of age. The incidence of coupling dropped off sharply with age, reaching levels of 30-40% between 10-18 days and occurring in less than 25% of injected adult neurons. Most coupled aggregates were arranged radially and somata were often widely separated and showed differing intensities of staining. The number of neurons per coupled aggregate also decreased dramatically as the cortex matured. Whereas 3-7 coupled neurons were commonly seen in cortex of 1-4 days, aggregates of more than 2 neurons were exceptionally rare in adults. The frequency of neuronal coupling did not vary with subpial depth at any age. Control extracellular ejections of dye did not stain neurons, and changes in coupling frequency were not due to changes in the amount of injected dye per unit cellular volume. When slices were bathed in low Ca^{2+}, Mn^{2+}-containing solutions to block chemical synaptic activity, short latency depolarizing potentials could be antidromically evoked in most neonatal neurons. Many of these potentials could not be blocked when they were elicited at short latencies after directly evoked spikes in a collision paradigm. They also persisted during somatic hyperpolarizations. Short latency depolarizations were much less frequent in adult cortex. These potentials probably represent electrotonically conducted spikes from coupled neurons. The results suggest that interneuronal coupling is extensive in the very immature neocortex, but that it declines just as the numbers of chemical synapses begin to multiply rapidly. We speculate that the dye and electrical coupling indicate the presence of gap junctions and that coupling may play some formative role in the early maturation of neocortical organization.

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121.12 SPECIFICITY IN TOPOGRAPHY OF HAMSTER PYRAMIDAL TRACT NEURONS DURING DEVELOPMENT. J. Kassel and K. Kalil. Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

Pyramidal tract neurons in the adult hamster are topographically organized within layer V of the sensorimotor cortex, such that the lumbar representation is posteromedial and the cervical area lies rostral and lateral to this. Previous developmental studies (Reh & Kalil, '81) demonstrated that this broad topography is established prior to the formation of corticospinal connections.

In previous experiments HRP was used to label cervical and lumbar projecting corticospinal neurons when their axons were respectively just invading the cervical dorsal horn (5 days postnatal) or still in the dorsal column at lumbar levels (8 days postnatal). Although the cervical and lumbar neurons occupy relatively discrete cortical areas similar to the adult, there is some overlap of these neurons in both infants and adults. However, with the HRP methods it was not possible to determine whether this overlap resulted from intermingling of neurons or from branching of single corticospinal axons to both cervical and lumbar spinal cord. Hence we employed double labeling techniques with fluorescent dyes to examine the degree of collateralization in corticospinal neurons at different stages of development. Infant hamsters received lumbar cord injections of Fast Blue at 8 days of age followed by injections of Nuclear Yellow at 12 days. Juvenile (18 days) and adult hamsters received cervical Fast Blue and lumbar Nuclear Yellow injections. Only those animals with no damage to fibers of passage in the dorsal columns were included in the results.

As with the HRP methods, the fluorescent dyes revealed that cervical and lumbar areas are well defined in the infant cortex, but also that a zone of overlap exists. This zone decreases with age and consists primarily of intermingled single-labeled cells. Double-labeled cells, i.e., with collaterals to both cervical and lumbar cord, were found along the lumbar-cervical boundary in the cortex. Although more numerous in infants than adults, they represent less than 2% of the labeled neurons even in infants. The scarcity of double-labeled cells early in development suggests that cortical topography is not significantly sharpened by loss of axon collaterals initially branching to widely separated segments of the spinal cord. Unless such collaterals are withdrawn before they are labeled, it is unlikely that corticospinal specificity is achieved by competitive interactions at spinal targets resulting in the loss of widely branching collaterals. We are currently investigating whether these developmental mechanisms could sharpen specificity among closely spaced spinal segments. (Supported by NIH grant NS-14428.)

121.13 ONTOGENETIC DEVELOPMENT OF NORADRENERGIC INNERVATION OF VASOPRESSIN-DEFICIENT NEURONS IN THE BRATTLEBORO RAT.
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The homozygous Brattleboro (DI) rat is characterized by a congenital defect in the genetic mechanism for vasopressin synthesis and manifests a profound diabetes insipidus. Anatomically, the supraoptic and paraventricular nuclei contain a normal complement of oxytocin-positive and vasopressin-free neurons. Because the neurons are still present in these nuclei this genetic mutant presents a useful model for the analysis of the ontogeny of neuron interactions. Specifically, the normal rat is characterized by an exceptionally dense noradrenergic innervation of vasopressin neurons (McNeill and Sladek, J. Comp. Neurol. 193: 1023-1033, 1980); this pattern is markedly depressed in the adult DI rat (Schöler and Sladek, Science 214:347-349, 1981) which raises a question concerning the establishment of the innervation pattern. Moreover, we have shown previously in normal rat that the vasopressin and oxytocin neurons appear to synthesize their respective peptides prior to the ingrowth of noradrenergic fibers (Khachaturian and Sladek, Peptides 1:77-95, 1980). In order to determine if vasopressin, or its associated neurophysin, is necessary either for the development or maintenance of a normal noradrenergic innervation, we examined pre- and postnatal DI rats from timed pregnant, homozygous mothers at 19 days prenatal and 1, 7, 14, and 21 days postnatal. Brain samples were prepared for the co-localization of monoamines and neuropeptides. Sections were examined for formaldehyde-induced histofluorescence, adjacent sections were immunohistochemically stained for rat neurophysin as a marker for oxytocin neurons; vasopressin-free neurons were stained with cresyl violet.

Like the normal rat, the vasopressin-free neurons of the Brattleboro rat received incoming noradrenergic fibers at 7 days postnatal; this pattern increased at 14 days and then appeared to remain stable or slightly decrease at 21 days. This latter observation is unlike the pattern seen in normal rat which continues to increase until reaching adult-like densities at 28 days. At all three postnatal ages, appositions between noradrenergic varicosities and peptide-free neuronal perikarya were seen. Presumably, this pattern continues to diminish past 21 days in order to assume the depressed pattern seen in adult DI rats. Thus, it appears that vasopressin or its associated neurophysin is not essential for the development of the noradrenergic innervation of the supraoptic nucleus; however it may be important for the maintenance of an appropriate innervation. Supported by USPHS Grant NS 15816 and AG 00847.

121.14 DEVELOPMENT OF HIPPOCAMPAL AFFERENT LAMINATION: SPECIFICITY APPEARS RELATED TO TRANSMITTER TYPE.
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Cholinergic septal and striatal tissues implanted into the entorhinal cortex will survive and reinnervate the hippocampal formation in neonatal rats, recreating the lamination patterns characteristic of native cholinergic fibers. It is not known whether this reinnervation is transmitter specific, or is produced by neurons of other transmitter type. In order to evaluate the hypothesis that the lamination is coded by a characteristic related to transmitter type, serotoninergic (5-HT) neurons derived from the raphe nucleus were implanted in the entorhinal cortex, and the pattern of reinnervation was compared to that of striatal or septal neurons.

The entorhinal cortex was ablated and the fimbria cut in 3 day old neonate rats. Three days later (day 6) the raphe nuclear area from rats of embryonic age 16-19 days was implanted into the entorhinal cortex lesion site. Control animals received both a fimbrial and entorhinal cortex lesion, but did not receive a raphe implant. Normal, unoperated littermates also served as control animals, and were used to determine the normal 5-HT innervation of the hippocampal formation. The tissue sections were processed for the indirect immunofluorescent localization of 5-HT immunoreactivity using an antibody directed against 5-HT. Additional sections served as absorption controls.

At 30 days post-implant, 80% (n=15) of the implants had survived in the entorhinal cortex site, and had reinnervated the hippocampal formation. In normal, unoperated rats the 5-HT immunoreactive fibers were laminated, with a dense 5-HT fiber network in the infragranular zone of the dentate gyrus, and a sparse but uniform, distribution of 5-HT fibers throughout the molecular layer. After the raphe implants, 5-HT immunoreactive fibers were uniformly distributed in the hippocampus and dentate gyrus, showing no apparent lamination pattern. The overall 5-HT innervation in the hippocampus and dentate gyrus was more dense after the raphe implants than in the normal animals. The control animals showed only a few, or a total lack of, 5-HT immunoreactive fibers. A lack of lamination patterns and the density of the 5-HT immunoreactive fibers after the raphe implant into the entorhinal cortex suggests that the 5-HT fibers are innervating vacant sites in the hippocampal formation. However, it remains to be seen if the 5-HT fibers are also restoring functional synapses to the deafferented sites in the hippocampus and dentate gyrus.

The pattern of the 5-HT innervation clearly differed from that of the cholinergic implants. Thus, a characteristic related to transmitter type appears to play a role in the specificity of the pattern of implant outgrowth in the developing rat dentate gyrus.

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121.15 EVIDENCE FOR A TRANSITORY CORTICOSPINAL PROJECTION FROM THE VISUAL CORTEX DURING EARLY POSTNATAL DEVELOPMENT IN THE RAT.
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It is now well established that during the early postnatal development of the neocortex of both rats and cats the initial widespread distribution of callosally-projecting neurons becomes progressively restricted within the tangential plane of the cortex. Further, it has been shown that this restriction is not primarily due to neuronal death but is brought about mainly by the elimination of axon collaterals. In order to determine whether a spatial restriction by collateral elimination is a general feature of cortical development or is unique to the callosal projection we have studied the distribution of the cells of origin of the pyramidal tract during the postnatal development of the rat neocortex.

During the first postnatal week, injections of true blue (0.3-0.5 µl of a 2-5% solution) into the pyramidal decussation result within three days in the labeling of pyramidal tract neurons in layer V which are distributed throughout virtually the entire tangential extent of the neocortex. Conversely, after comparable injections during the fourth postnatal week, or in adult animals, the distribution of such cells is much more restricted. This developmental restriction is most dramatic in the occipital cortex; during the first postnatal week the band of retrogradely labeled pyramidal tract neurons extends into and is spread throughout the visual cortex, but no pyramidal tract neurons are seen in this area in the adult. When similar true blue injections are made into the pyramidal decussation during the first postnatal week and the animals are allowed to survive until the fourth week (by which time restriction of the projection has occurred) the distribution of labeled neurons in the cortex is as widespread as in the immediate postnatal period and includes the visual cortex. Thus many of the layer V neurons in the occipital cortex which initially send a collateral into the pyramidal tract remain viable several weeks later, but no longer maintain a pyramidal tract axon.

This transitory corticofugal projection from the visual cortex was labeled in five day old rats by injections of either ^{3}H-proline or WGA-HRP into the occipital cortex; the animals were killed 12-24 hours later and processed for autoradiography or by the TMB histochemical method, respectively. In these cases (but not after comparable injections in adult rats) labeled fibers were seen in the pyramidal decussation and within the contralateral dorsal column as far caudally as the lower cervical segments of the spinal cord. No unequivocal labeling has been seen in the spinal gray matter.

These findings indicate that the elimination of axon collaterals may be a general feature of the development of cortical projection systems and that such transitory collaterals may traverse considerable distances; but they leave open the question of whether such collaterals establish transitory synapses. (Supported by NIH grants NS-16980 and EY-03653)

121.16 NON-SELECTIVE REGENERATION OF THE SYMPATHETIC INNERVATION OF THE PINEAL GLAND AFTER CRUSHING THE INTERNAL CAROTID NERVES.
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The enzyme serotonin N-acetyltransferase (NAT) in the rat pineal gland has a large circadian rhythm with peak activity occurring at night. The rhythm in enzyme activity is dependent on stimulation of the pineal gland by neurons in the superior cervical ganglia via the postganglionic internal carotid nerves (ICN). Two days after both ICN were cut, crushed, or frozen in adult rats, night-time NAT activity was decreased by 90%. The remaining low level of enzyme activity was not affected by bilateral decentralization of the superior cervical ganglia, indicating that it did not depend on the activity of a few superior cervical ganglion neurons which might innervate the pineal gland by a nerve trunk other than the ICN. The neuronal uptake capacity of the pineal for norepinephrine was totally abolished by bilaterally lesioning the ICN, further indicating that the sympathetic innervation of the gland had been destroyed.

Three months after bilaterally crushing the ICN, night-time NAT activity was still only 17% of control values (6.2 ± 1.2 compared to 37.3 ± 3.5 pmol/µgm per 20 min), though in these animals bilateral decentralization of the superior cervical ganglia produced a decrease in the NAT activity to 0.28 ± 0.05 pmol/µgm per 20 min. Thus, 3 months after the ICN lesions, NAT activity was again dependent on sympathetic nerve stimulation. In contrast to the rather small recovery of NAT activity, the norepinephrine uptake capacity of the gland had recovered by 60%. A similar discrepancy between the extent of recovery of NAT activity and NE uptake was observed when the ICN were frozen rather than crushed.

To determine whether, in these lesioned animals, the sympathetic nerves which had reinnervated the pineal gland were capable of regulating NAT activity, their cervical sympathetic trunks were stimulated electrically at 5 Hz for 3 h during the day-time. NAT activity increased in these animals, as it did in sham operated animals, from low day-time values to near peak night-time values. Thus the sympathetic nerves reinnervating the pineal gland are capable of increasing NAT activity to high nocturnal levels when electrically stimulated and yet these animals do not recover a normal NAT rhythm. We conclude that following bilateral lesioning of the ICN, the pineal gland is reinnervated by different sympathetic neurons than those which had previously innervated this tissue and that these neurons do not receive the type of neural information from the central nervous system which is necessary for regulating a normal circadian rhythm in NAT activity.
Supported by USPHS grants NIH NS 17512, NS 07009, and MH 00162.

122.1 INCREASED SYNAPTIC DENSITY IN HIPPOCAMPUS OF AGING RATS CHRONICALLY TREATED WITH A NEURAL STIMULANT. P.W. Landfield, M.D. Applegate* and T.A. Pitler* Dept. of Physiol. and Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103

We recently reported that long-term (10 mo.) treatment of aging rats with daily administration of a neural stimulant (pentylenetetrazol-PTZ) or of a behaviorally-active analog of $ACTH_{4-9}$ (ORG 2766) reduced the development of some light microscopic correlates of aging in the hippocampus (e.g., neuronal density and glial reactivity changes were reduced in treated animals sacrificed at 27 mo. of age). (Science, 214: 581, 1981). These morphologic patterns were similar to, but not precisely the same as, patterns we found following long-term adrenalectomy (ibid and Soc. Neurosci. abstracts, 1979). The data therefore suggested that the adrenalectomy-induced rise in ACTH (and possible resulting neural stimulant effects) may contribute to some, but not all of the reduction of brain aging correlates seen with adrenalectomy. Concomitantly, it was found that the drug-treated aged rats performed better on a maze-reversal learning task than did untreated controls of the same age. Young rats also performed better than untreated aged rats.

In this report we summarize recent findings from quantitative electron microscopic analyses of material from some of the same animals. Ultrathin sections were cut from a well-defined region of field CA1, and a series of micrographs were randomly shot (x 15,000) across the face of the section (in the apical dendrites, 200 m from the pyramidal cell somata) and printed at a final magnification of x 40,000. Five PTZ-treated aged animals, 11 saline-injected aged controls, 4 young-mature (8 mo.-old) rats, and 7 chronically adrenalectomized aged rats were studied with E.M.

The micrographs were quantified blind for synaptic density, axon terminal area, and length of postsynaptic densities, among other variables. With age, we found a decrease in synaptic density and an apparent increase in mean area of axon terminals. Adrenalectomy prevented the terminal area increase, but not the decrease in synaptic density. Conversely, PTZ did not block the terminal area increase but did block the synaptic density decrease. Synaptic density was correlated with maze learning performance across groups and among aged controls.

These data are consistent with the view that chronic neural stimulation may exert an influence (trophic?) on the adult brain that reduces or counteracts brain aging processes, while adrenalectomy may reduce brain aging correlates by slowing subtle aspects of continued growth. However, further work is need to examine the generality and implications of these findings. (Supported in part by AG 01552)

122.2 DENDRITIC CHANGES IN AGING MACACA MULATTA. E. Uemura, D. Baker*. Dept. of Veterinary Anatomy, Iowa State Univ., Ames, IA 50011.

The dendritic branching pattern was studied in the subiculum of nine Macaca mulattas from 7 to 28 years of age. The three middle-aged animals (18, 19, 20 years old) showed the highest mean apical dendritic branches (41.7/neuron) and mean total dendritic length (3,744 µm/neuron). The three young animals (7, 10, 12 years old) showed 32.9 branches and dendritic length of 3,064 µm per neuron, whereas the three old animals (27, two 28 years old) showed 28.9 branches with a dendritic length of 2,807 µm. The basal dendritic branch was similar for the young and middle-aged animals with mean number of 33.4 and 34.6 branches per neuron, respectively. However, a prominent increase (11%) in dendritic length was found in the middle-aged animals (3,109 µm). The three oldest animals showed a significant reduction in the basal terminal branches (30.2 branches/neuron) and in their length (2,250 µm). Frequency distributions showing the number of measurements within each 10 µm increment per dendritic order, in both the centrifugal and centripetal ordering method, further substantiated age-related changes in the dendritic tree. There was continued branching and growth of the apical dendrites in adulthood. Basal dendrites did not show any added complexity, but rather showed continued growth of existing terminal branches. The three oldest animals showed a preferential loss of whole terminal branches on the apical portion of the dendritic tree, whereas shortening of existing terminal branches was the characteristic feature of the basal dendrites.

122.3 SYNAPSE NUMBER AND SPINE VOLUME IN DENTATE GYRUS OF AGED RATS. C.A. Curcio and J.W. Hinds. Dept. of Anatomy, Boston University School of Medicine, Boston, MA 02118.

Anatomical (Geinisman and Bondareff, '76) and physiological (Barnes and McNaughton, '79, '80) studies have suggested a loss of perforant path synapses in the outer 2/3 of the molecular layer (OML) of the aged rat dentate gyrus, although this finding has not been confirmed by others (Cotman and Scheff, '79). Because the hippocampus may continue to grow in mature animals (Diamond et al., '75), it is not known how the number of synaptic profiles per unit area (Na) of OML reflects the absolute number of synapses. In order to address more directly the question of age-related synapse loss independent of tissue volume, we are studying the number of axospinous synapses per dentate granule cell in aged rats.

Changes in spine size and shape have been proposed as structural correlates of enhanced synaptic transmission following high frequency stimulation of the perforant path (Fifkova and Van Harreveld, '75) and of the Schaffer collateral system (Lee et al., '80). Because of reported increases in synaptic efficacy (Barnes and McNaughton, '79, '80) in the aged rat dentate gyrus, we are also studying spine volume as a function of age in the same tissue.

Blocks of aldehyde-perfused hippocampus from Sprague-Dawley rats aged 4.5-6, 24, and 29.5 months (50% survival = 27 months) were prepared for electron microscopy using standard techniques. The numerical density (Nv) of axospinous synapses (and by inference, Nv of spines) was determined from strips of electron micrographs spanning the OML. The volume fraction (Vv) of spines was measured by point-counting techniques from the same micrograph strips. Nv of dentate granule cells and the heights of the granule cell layer and OML were determined from semi-thin sections through the same blocks. From these measured parameters it was possible to derive the number of OML synapses per granule cell and mean spine volume.

Data are being collected from coded tissue and will be presented.

122.4 AGE RELATED EFFECTS ON GLIAL-NEURONAL RATIOS IN FELINE SPINAL CORD Francisco Chávez-Almanza*, Anne S. Kaplan and Arnold B. Scheibel. Department of Anatomy and Brain Research Institute., U.C.L.A. School of Medicine , Los Angeles, California 90024.

Structural microscopic alterations in neurons and glial elements due to the senescent process have been described. However there are no quantitative studies about the ratio of these cells in the spinal cord of aging animals. We wished to ascertain whether the relative number of supportive cells associated with medium size and giant neurons was modified by the aging process in cat.

The number of neurons, satellite oligodendrocytes, microglial cells and their ratios were studied in 3 young (2-3 years) and 4 senescent (17 years) cats. Sixty-three Nissl-stained longitudinal sections of the cervical enlargement of each cat were studied. Neurons of laminas VIII and IX were drawn at 400 X from each section with the aid of a camera lucida system. The gray area of each section was drawn at 4 X. The areas of these drawings were determined planimetrically. The satellite oligodendrocytes corresponding to giant neurons (greater than 80 µM in diameter) and medium size neurons (22-50 µM) were analyzed. The number of microglial cells around medium size neurons was also counted. Neuronal and glial counts are expressed per mm^2. The glial counts are expressed as a glia to neuron ratio. There were no significant quantitative differences between the two groups of animals.

		Giant	Medium	Medium
Young cats	S.O./A	2.33±0.87	2.29±0.90	M/A 1.93±0.66
Old cats	S.O./A	2.40±1.34	3.31±1.53	M/A 3.40±1.83
Young cats	S.O./N	0.89±0.17	0.60±0.19	M/N 0.50±0.12
Old cats	S.O./N	0.69±0.25	0.45±0.08	M/N 0.45±0.03
Young cats	N /A	2.55±0.56	3.80±0.55	3.80±0.55
Old cats	N/ A	3.35±0.93	7.55±3.94	7.55±3.94

S.O.=satellite oligodendrocytes
A =area (mm^2)
N =neurons
M = microglial cells
mean ± S.D.

It appears that the senescent process in the spinal cord does not affect significantly the number of satellite glial cells. Therefore the ratio between neurons and their corresponding satellite glial cells remain almost unchanged. During aging the structure and organization of the neurons and glia change. But their number in different parts of the CNS may increase, decrease or remain without change. These findings support the hypothesis that the phylogenetically older structures of the CNS are less affected by aging. (Frol'kis, V.V., et al., Aging of the central nervous system. Hum. Physiol. 4 (4) 478-499 Jul-Aug ., 1978). Supported by NIH grant AG-1754-03. F.Chávez-Almanza on leave from Depto. de Biología Humana E.N.E.P. Zaragoza U.N.A.M. and A.N.U.I.E.S. Mexico .

122.5 HISTOLOGICAL STUDIES OF AGING IN THE MEDIAL NUCLEUS OF THE TRAPEZOID BODY IN RATS. M. A. Casey. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

The medial nucleus of the trapezoid body (MTB) is the largest (i.e., contains the most neurons) cell group in the rat superior olivary complex. By relaying impulses from the contralateral ventral cochlear nucleus to the lateral superior olive, the MTB is believed to function in sound localization. Since changes in sound localization have been reported in aged rats (Harrison, J.M., Exp. Aging Res., 7(4):467, 1981), and since few studies have dealt with the aging brainstem, the present study was initiated to determine if morphological changes occur in rat MTBs with aging. Twenty-three Sprague-Dawely rats were studied at the following ages: 2-3 months (MO), 6 MO, 18 MO, and 24 MO. After perfusion with Bodian's fixative, brainstems were removed, dehydrated, embedded in paraffin, and cut serially through the MTB at 16 μm in the transverse plane. Every fourth section was mounted and stained with protargol for neuron counts and analysis of the giant synaptic endings (chalices of Held) on MTB neurons. Additional sets of paraffin sections were stained with thionin to differentiate neuronal sub-populations in the MTB.

A significant amount of neuron loss occurs in the rat MTB with aging ($p<0.001$). A 34% decrease in the mean number of MTB neurons was observed between 2-3 and 24 MO of age. As in cats (Morest, D.K., Brain Res., 9:288, 1968), three neuron types were observed in thionin preparations of the rat MTB: ∿82% of all neurons were somewhat rounded with eccentric nuclei (principal cells), ∿15% were elongate or spindle-shaped, and ∿3% were stellate with centrally-placed nuclei. The proportions of the three cell types did not vary with aging ($p>0.05$). In protargol-stained sections, ∿25% of MTB neurons were associated with visible chalices of Held in both young and old animals.

For examination of age pigment in semi-thin plastic sections, rats aged 3 and 24 MO were perfused with mixed aldehydes, and small brainstem slabs containing the MTB were osmicated, embedded in Araldite, sectioned at 2 μm and stained with toluidine blue. Similar to the medial superior olive in aged rhesus monkeys (Brizzee, K.R., J. Gerontol., 29(4):366, 1974), age pigment accumulation in rat MTB neurons is relatively sparse. The heaviest age pigment deposits were observed in glial cells of the MTB.

This is the first study to provide evidence of neuron loss in a non-human brainstem nucleus. Neuron loss in the rat MTB may be involved in the age-related changes in sound localization reported in the rat.

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122.6 FAVORABLE RESULTS WITH THE GOLGI COX BUT NOT THE RAPID GOLGI METHOD WHEN APPLIED TO AUTOPSIED HUMAN BRAIN. S.J. Buell*. Neurology Dept., University of Rochester, Rochester, NY 14642.

We recently reported quantitative evidence for dendritic growth in normal human aging but not in senile dementia based on observations of cerebral cortex obtained at autopsy and prepared by the Golgi Cox method. This finding was in stark contrast to previous reports of dendritic regression in human aging based on rapid Golgi-stained cerebral cortex. The Golgi Cox method is especially useful for study of entire dendritic trees because it gives reliable progressive, controlled impregnation of neuronal processes, staining dendrites very dark, and leaving the background very clear. It has been used in a number of other studies for such purposes. However, the method does not stain dendritic spines as clearly and as often as does the rapid Golgi stain. Thus, many others have used variations of the rapid Golgi method for consistent demonstration of dendritic spines and for examination of the general configuration and extent of dendritic trees in laboratory animals. In this report we present our findings comparing the results obtained when the Golgi Cox and five variants of the methods rapid Golgi were used on tissues taken from the same individual human cases at autopsy. Adjacent blocks of hippocampal formation and precentral gyrus from nine cases were prepared by the two methods. The cases ranged in age from 39 to 99 years, had postmortem times ranging from 6 to 28 hours and included cases of dementia of the Alzheimer type. Without exception, the methods produced very different results. The Golgi Cox method resulted in impregnation of many neurons with rich dendritic plexuses and normal overall appearance. Occasional cells appeared grossly atrophic with irregular somata and apparent loss of apical and basilar dendritic segments. With the rapid Golgi method, far fewer cells were impregnated. Of those which were impregnated, the vast majority exhibited grossly atrophic features while few, if any, impregnated neurons had rich dendritic plexuses or were otherwise normal in appearance. Such striking differences between the methods were observed in all laminae of the precentral gyrus, granule cells, the dentate gyrus, pyramids of Ammon's horn and all laminae of the parahippocampal gyrus. Thus, the rapid Golgi method appears to be highly sensitive to postmortem delay or other factors which accompany studies involving human brain tissues obtained at autopsy. The Golgi Cox method appears to be relatively insensitive to such factors.

122.7 INTRACELLULAR ANALYSIS OF MOTONEURON PROPERTIES IN AGED CATS. P.A. Boxer*, F.R. Morales*, S.J. Fung and M.H. Chase. Depts. of Physiology and Anatomy and the Brain Research Institute, Sch. of Medicine, University of California, Los Angeles, CA 90024

Lumbar motoneurons in the cat have long been favored cells for the elucidation of basic neuronal properties, and a wealth of normative data is available regarding the functioning of these neurons in adult animals. We were therefore interested in determining the possible impact of age on various electrophysiological characteristics of motoneurons. Experiments were performed on age-documented animals; five old cats, 14 to 15 years of age and four adult controls, 1 to 3 years of age. All animals were maintained under sodium pentobarbital anesthesia in a standard acute cat preparation. Intracellular recordings were obtained from identified hindlimb motoneurons with microelectrodes filled with 3M KCl. The resting potential level and action potential amplitude were similar in these two groups of animals (-66 mV and 78 mV for the resting and action potential, respectively, in the old cats, versus -68 mV and 77 mV in the adult animals). There was a statistically significant decrease in the mean conduction velocity in the old cats (68 meters/sec) compared to the mean conduction velocity in the adult control cats (89 meters/sec), confirming our previously reported results. The duration of the afterhyperpolarization (AHP) was measured following action potentials elicited by (1) intracellular stimulation with brief depolarizing current and/or (2) antidromic activation following stimulation of the appropriate muscle nerve. The mean duration of the AHP in the old animals was 87.6 ± 35 msec, compared with 71.4 ± 21 msec in the adult control animals. This difference was statistically significant ($t=3.3$, $df=151$, $p<0.001$). We conclude that the alteration in AHP duration is not secondary to changes in the resting potential or action potential amplitude, but is rather a consequence of senescence.

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122.8 EFFECTS OF AGING ON NEUROMYAL TRANSMISSION IN INBRED CBF-1 MICE. Hirashi Hasuo* and Alexander G. Karczmar. Dept. of Pharmacology, Loyola University Medical Center, Maywood, IL, 60153.

This study concerns the effect of age on neuromyal transmission in CBF-1 mice that were inbred and maintained as colonies of aging animals (Hill, Charles River Digest, 20:1, 1981). One year old (mature) and 2 years old animals were compared. Isolated phrenic nerve - diaphragm preparations were employed and microelectrodes were used to measure resting membrane potential (RMP), frequency of miniature endplate potentials (MEPP's), endplate potentials (EPPs) and the quantal content (QC). Significant differences were found between the two age groups with respect to RMPs, MEPP frequency and QC. RMP values were -76.37 ± 3.29 mv {n(number of cells studied)=51} and -74.54 ±3.72 mv (n=65) for mature and old mice, respectively ($p<0.01$); MEPP frequencies were 1.97 ± 1.15/sec (n=46) and 1.28 ± 0.59/sec (n=41), for mature and old mice, respectively ($p<0.001$); QC values that were obtained by means of the failure method were 1.58 ± 0.58 (n=31) and 1.05 ± 0.38 (n=45) for mature and old mice, respectively ($p<0.001$). The values for MEPP amplitude were not significantly different for mature (0.934 ±0.344 mv, n=20) and old (1.001± 0.324 mv; n=34) mice. Preliminary data suggest that the EPP rise time did not differ between mature and old mice while the EPP decay time was shorter for the aged population. These results indicate that presynaptic activity, both spontaneous and evoked, decreases with age and that changes in postsynaptic membrane also occur with age. There are relatively few studies of age effect on neuromyal transmission, none that concern inbred aging mice populations. The decrease of MEPP frequency with age was also noticed in the rat by Gatmann et al. (J. Physiol., 219:331, 1971), but opposite effect was reported by Smith (Exp. Neurol., 66:650, 1979). Our data suggest that even when the number of cholinergic cells does not decrease with age (as may be the case in CNS), their capacity to release ACh diminishes. Supported in part by BRSG Grant No. 104. CBF-1 mice were kindly supplied by NIH Institute on Aging.

122.9 STRAIN DIFFERENCES IN AGING OF RAT HIPPOCAMPUS. S. T. DeKosky, S. W. Scheff and C. Hackney*. Lexington VA Medical Center, Depts. of Neurology and Anatomy, and Sanders-Brown Research Center on Aging, Univ. of Ky. Coll. Med., Lexington 40536.

Establishment of general neurobiological principles of aging in rat brain depend upon the assumption that different strains of rats age in a similar fashion. We have examined changes in the hippocampus over the life span of two common strains of rat, the Fischer 344 and Sprague-Dawley (SD). Both total brain weight and whole hippocampal wet weight increased progressively and significantly over the life span of the Fischer 344 (3, 9, 16, 21, 28 months), whereas no increment in either brain weight or hippocampal weight was noted in SD rats. In an effort to determine what tissue component might be responsible for the progressive increase in hippocampal weight, we examined structural membrane markers in freeze-dried transverse sections of hippocampus from 3, 16, and 28 month-old Fischer 344 and SD rats. Hippocampal DNA per dry weight (cell packing density) of both Fischer and SD males decreased significantly between 3 and 16 months. DNA per dry weight in senescence, however, was not significantly different from 3-month controls. Sialoganglioside, a neuronal membrane marker, decreased per dry weight in the hippocampus of both strains. The decline was more rapid in the Fischer 344, but by 28 months a 22% loss had been sustained in the Fischer 344's and the SD's. Galactocerebroside, a quantitative index of myelin, did not change in the hippocampus of the SD between 3 and 28 months, but in the Fischer 344 a 20% increase of galactocerebroside per dry weight was found. Thus, both strains show a decrease in neuronal membrane over the life span, with this loss occurring at different rates. The hippocampi of both strains lose cells by middle age, but animals which survive to senescence have total cells equal to that of their 3 month-old controls. Continuous myelination appears to be responsible for the progressive age-related increase in weight of the Fischer hippocampus; both wet weight and myelin content of the SD hippocampus remain stable. These data suggest that alteration or loss of neuronal membrane is a general characteristic of aging rat hippocampus, but that major differences exist in response of other lipid membranes to the aging process. Supported by the VA Medical Research Service and NIH grants NS0444 and NS16981.

122.10 ALTERATION OF BETA-ADRENERGIC RECEPTORS IN AGING RAT CORTEX. R. G. McAllister*, S. T. DeKosky and T. G. Tan* (SPON: R. Miller). VA Medical Center and Depts. of Medicine and Neurology and Sanders-Brown Research Center on Aging, Univ. of Kentucky College of Medicine, Lexington, Ky. 40536.

Age-related alterations in β-adrenergic receptors have been reported in different areas of the rat brain, and changes in adrenergic responsiveness in aging may be associated with such decrements. There is a significant regional variation in the characteristics of β-adrenergic receptors, and the relative proportion of β_1 and β_2 receptors differ in various areas of brain. We examined possible regional differences in β-adrenergic receptors and evaluated changes in affinity (K_d), receptor density (R_D) and receptor subtype in freshly dissected samples of male Fischer 344 rat brain. At 3 and 24 months of age, cortex, hippocampus and cerebellum were analyzed for binding, utilizing ^{3}H-dihydroalprenolol (DHA) at concentrations from 0.25-15nM. 10^{-5}M d,l-propranolol was used as inhibitor. Binding was stereospecific and saturable. Scatchard analysis of the binding data revealed linear plots, indicating a single class of binding sites, in the hippocampus (K_d=4.8nM; R_D=209.4 fmol/mg protein) and cerebellum (K_d=0.7nM; R_D=37.2 fmol/mg protein). No differences in affinity or receptor concentration were seen in either area in aged animals. However, Scatchard analysis of the binding in young Fischer cortex indicated two populations of receptors. One of these was a high affinity (K_d=0.6nM), low density (R_D=68.5 fmol/mg protein) receptor population comprising approximately 25% of the total sites. The other receptor population had lower affinity (K_d=4.3nM) and higher density (161.3 fmol/mg protein) and comprised 75% of the binding. Scatchard plots of senescent cortex indicated only one type of β receptor, which was similar in density and affinity to the low affinity, high density population seen in the young rat cortex. When the selective β_1 antagonist metoprolol was employed as inhibitor (10^{-5}M) of DHA binding in young rat cortex, the low affinity, high density population was not found, identifying these receptors as β_1. Furthermore, the K_d (0.6nM) of the high affinity, low density receptors, which were lost in aging, was similar to that of the β receptors in the cerebellum, known to be almost entirely of the β_2 subtype. These data suggest that the β_2 population of adrenergic receptors may selectively decline in the cortex of the aging rat. Supported by the VA Medical Research Service and NIH grant NS0444.

122.11 AGE-RELATED CHANGES IN RECEPTOR SENSITIVITY: RECEPTOR SUBTYPES AND CALMODULIN. C. C. Loullis, D. I. Benson, R. T. Bartus, L. R. Meyerson, J. Rotrosen and A. S. Lippa. Dept. CNS Research Medical Research Division of American Cyanamid, Lederle Labs, Pearl River, NY 10965 and Manhattan VA Medical Center, New York NY 10016

Based on the ability of muscarinic receptor antagonists to produce memory disturbances similar to those observed in aged subjects, it has been proposed that alterations in brain muscarinic cholinergic mechanisms may be responsible for the memory disturbances of the aged. More specifically, considerable interest has recently been focused on neurotransmitter receptors as possible sites of cellular malfunction which may be responsible for the behavioral changes which occur during aging. In previous studies it was demonstrated that large functional disturbances in postsynaptic muscarinic mechanisms occurred in aged animals exhibiting small decreases in muscarinic receptor density while exhibiting significant memory impairments. Single cell recordings revealed a large and specific decrease (>60%) in sensitivity to iontophoretically applied acetylcholine in hippocampal pyramidal cells in the aged brains. A possible explanation for the relative differences in degree of change in neuronal activity and behavior versus muscarinic receptor density is that the loss of a small number of functionally distinct subpopulations of muscarinic receptors may have produced extensive physiological consequences. Biochemical evidence for such heterogeneity of muscarinic receptors has been reported. Another possibility might involve calmodulin (a small thermostable, acidic, calcium binding protein). Changes in calmodulin have recently been associated with receptor responsiveness, probably linked to a calcium dependent post- receptor process. In order to test these two hypotheses, dorsal hippocampal and cortical tissue from three groups of animals (5, 12 and 24 mos. old) was dissected on ice and stored at -20°C for subsequent determinations of atropine and oxotremorine displacement of ^{3}H-QNB binding and calmodulin levels. Atropine displacable ^{3}H-QNB analysis in the dorsal hippocampus revealed a small (16%) decrease in total receptor binding in old animals consistent with previous findings. Interestingly, Hofstee plots of oxotremorine displacement of ^{3}H-QNB revealed a 67% decrease for the low affinity sites and no change in high affinity sites in the aged (24 mo) group. No significant age-related changes in calmodulin levels were found. These results support the hypothesis that changes in a functionally distinct subpopulation of muscarinic receptors in the hippocampus may account for the large age-related functional alteration in neuronal activity and behavior. Is is also noteworthy that the low affinity muscarinic sites are presumably coupled to a transducer system whereas the high affinity sites are not. This may indicate that changes in post-receptor processes (other then calmodulin) may be influential. Further studies are in progress to carefully evaluate this premise.

122.12 **SEX-DEPENDENT ALTERATIONS IN CENTRAL NERVOUS SYSTEM (CNS) CHOLINE-O-ACETYLTRANSFERASE (ChAT, EC 2.3.1.6) DURING AGING.** Cézanne Garcia*, Nancy J. Woolf and Larry L. Butcher. Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA, 90024, U.S.A.

ChAT activity was measured (method of Fonnum, F., J. Neurochem., 24:407, 1975) in 23 regions of the CNS in young adult (11 months old) and aged (35 months old) male and female rats of the Fisher 344 strain. Although there was a general tendency for enzyme activity to show decrements with increased age in most of the neural regions analyzed, none of these trends were statistically significant when data from both sexes were pooled. When considered separately, however, ChAT activity appeared affected by the aging process to a greater extent in males than in females. The most striking difference was found in the cerebral cortex. Males showed a 20% loss of cortical ChAT activity ($p<0.05$) whereas the decrement in females was only 2% and not statistically significant. Both sexes displayed slightly decreased enzyme activity in the caudate-putamen complex and nucleus accumbens, but, in the hippocampus, males showed a significant reduction in ChAT activity whereas females showed a tendency for increased activity in that structure. [Support: USPHS AG-01754].

122.13 AGE-RELATED CHANGES IN CHOLINE UPTAKE AT THE RAT NEUROMUSCULAR JUNCTION. D.O. Smith and C.T. Gibson*. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

The choline uptake system has been studied at the neuromuscular junction of the diaphragm muscle in rats aged 10 and 28 mos. Accumulation of [^{3}H]-choline was assayed in innervated and non-innervated tissue; uptake by the phrenic nerve terminals was estimated by calculating the difference between these two measurements. A high-affinity and a relatively nonsaturable component were identified. Uptake was found to be linear in both innervated and noninnervated tissue for at least 5 min following addition of 1-μM labeled choline. The amount taken up in the presence of either HC-3 or Na^+-free saline was reduced to values ranging from 11% to 41% or 26% to 73%, respectively, of the values measured in normal saline. Accumulation was not saturable for choline concentrations ranging from 0.25 to 12.0 μM. Thus, kinetic data were obtained using the rate equation for a non-saturable process. In both age groups, K_m values of the terminals were 0.9 μM. The values of V_{max} were 0.9 and 0.6 pmol/4 min/fiber for the young and the aged rats, respectively; this difference is statistically significant. The slope of the non-saturable component was also less in terminals of aged animals by an amount which is statistically significant. It is concluded that there is less choline uptake in the older rats and that this is due primarily to a reduced number of transport sites.

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122.14 AGE-RELATED CHANGES IN THE END-PLATE ARCHITECTURE OF FUNCTIONALLY DIFFERENT MUSCLES IN RATS. J.L. Rosenheimer and D.O. Smith. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

The architecture of end-plate regions in extensor digitorum longus (EDL) muscle and soleus muscle was examined in control (10 mos) and aged (28 mos) rats following cholinesterase staining of the end plate and silver-gold impregnation of the axon and its terminal arborization. The results were compared to those obtained in a similar study of the diaphragm muscle.

The average number of nerve terminal branches per EDL end plate was 12 in both age groups. In soleus end plates, the corresponding values were 15 and 17 in control and aged rats, respectively. This difference was only moderately (0.1 level) significant statistically. This is opposed to a significant (0.05 level) difference in the average number of terminal branches observed in control and aged rat diaphragm end plates (13 and 18, respectively).

The number of myelinated motor axon branches projecting into the end plates increased with age in the EDL; it did not change with age in the soleus or diaphragm. This indicates that while the increased number of terminal endings in the soleus and diaphragm end plates of older animals is due to more frequent branching within the end-plate region, terminal branching occurs less frequently with age in the EDL.

There was also a significant increase with age in the size of the end-plate region of the EDL and soleus muscles. This is contrary to the diaphragm muscle, in which there were no age-related changes in end-plate size.

Indications of terminal branch degeneration were observed in 10% and 13% of control and aged rat EDL muscles and in 28% and 15% of soleus muscles, respectively. This was not accompanied by significant differences in the occurrence of terminal or nodal sprouting in either muscle. These data are contrary to those observed previously at the diaphragm neuromuscular junction, where both processes appeared to decrease with age. In 54% and 29% of EDL and soleus end-plate regions of aged rats, respectively, ultraterminal sprouting was observed. These did not terminate in a region which stained for cholinesterase. This was a significant increase relative to control animals in the EDL muscle only.

It is concluded that the EDL, a hind limb fast twitch muscle, changes differently as a result of aging than the soleus, a slow twitch postural muscle. Both limb muscles appear to be more severely affected than the diaphragm, a vital muscle in respiration. This could be influenced by changes in the frequency of use with age.

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122.15 CHRONIC DIETARY CHOLINE MODULATES SYNAPTIC PLASTICITY IN THE CEREBELLUM OF AGING MICE. C. BARTONI-FREDDARI* and R.F. MERVIS. V. Verzar Intl Lab for Exp Gerontology, Italian Section, Ctr. for Cytology, INRCA, Ancona, ITALY and Dept. Pathology (Neuropathology), The Ohio State University Medical Ctr., Columbus, OH 43210

An EM investigation was carried out to evaluate morphometric parameters of ethanolic phosphotungstic acid (E-PTA) - stained synapses in the cerebellar glomeruli of mice (C57Bl/6J) which had been maintained on chronic (11 month) choline-enriched (ChE), choline-deficient (ChD) or control (BC) diets. The dietary regimen was started when the mice were approximately 8 months-old and continued until they were sacrificed when 19 months-old. In a double blind procedure, E-PTA stained synapses were evaluated from photomicrographs. The numerical density (Nv) and the average length ($\bar{L}$) of synaptic contact zones were measured. These values provided a measure of the surface density (Sv) (representing synaptic area) of the contract zone. Analysis showed that the total surface density of the synaptic contact zone (Sv) decreased with normal aging. This decrease is attributed to a reduced number of synaptic contacts (Nv), since the remaining synapses showed an increase in average length ($\bar{L}$). In the chronic ChD mice not only was there a loss of synapses (Nv), but the overall synaptic surface density (Sv) was also reduced. However, the chronic ChE diet resulted in an increase in both the number of synapses (Nv) as well as total synaptic surface density (Sv). Moreover, although ($\bar{L}$) increased with normal aging, this parameter did not increase in the aging ChE mice. This study shares parallel findings with previous results obtained in the same mice at both behavioral and anatomical levels. Thus Bartus et al. (Science 209:301, 1980) had shown that after 4 1/2 months of the dietary regimens, in comparison to age-matched controls, the ChE mice showed improved -- and the ChD group, reduced -- retention of learning. Furthermore, Golgi studies of Layer V pyramidal cells in the fronto-parietal cortex of these same mice also demonstrated that chronic (11 month) ChE not only repressed normal age-related spine loss but also significantly increased the overall dendritic spine density. These studies imply that dietary choline enrichment maintains or enhances neuronal interconnectivity. The results of this EM study demonstrated one form of plasticity in the cerebellum of the aging CNS, i.e. that with synaptic loss (Nv) the remaining synapses exhibit a compensatory hypertrophy ($\bar{L}$). The chronic ChE diet appears to illustrate, perhaps, another form of plastic response. In these mice, an increased (Nv) and (Sv) unaccompanied by ($\bar{L}$) suggests that the chronic ChE diet could influence phospholipid synthesis which may, in turn, modulate neuronal membrane plasticity and thereby maintain the structural integrity of synapses in the aging brain.

122.16 CHOLINERGIC DRUG EFFECTS ON RECENT MEMORY IN AGED RATS. M. Ordy, G. Thomas and W. Dunlap, Pennwalt Pharm. Corp., Univ. of Rochester, Rochester, NY 14623, Tulane Univ., New Orleans.

Physostigmine has been reported to enhance, and scopolamine to impair recent or working memory. Aging has been reported to impair recent memory similar to scopolamine impairment of memory in young subjects. Various experimental strategies have been proposed to separate cholinergic drug and age effects on recent memory from concomitant effects on sensory function, learning, motivation, and motor coordination. State-dependent designs with post-learning administration of cholinergic drugs have attempted to differentiate between drug effects on learning and memory. However, sensory function, motivation, and motor performance operate not only during learning but also during memory testing, and these functions may change significantly during aging. In this study, the effects of physostigmine and scopolamine were examined on working memory and performance in young (4-6), middle age (10-12) and aged (30-34 month) rats. Working memory was tested in a discrete trial, paired-run, delayed alternation procedure in the "T-Maze" in which drug and age effects were also distinguishable from other sources of performance effects. In each pair of runs, rewarded forced runs provided left-right spatial information. Delayed (0, 45, 90 sec) win-shift, or choice runs to opposite location served as measures of drug and age effects on working memory. Start, choice, and goal box latencies comprised measures of performance. Significant age effects were observed in working memory, and in start, choice and goal box latencies. Physostigmine and scopolamine had opposite effects on start, choice, and goal box latencies without selective enhancement or impairment of working memory at doses used. Drug effects interacted with age effects on working memory and performance. Compared to saline controls, multivariate analyses indicated differential profiles for physostigmine and scopolamine on working memory and on performance among young, middle age, and old rats. Previous passive avoidance or appetive studies of drug and age effects on so-called "short-term" memory were conducted without discrete "working" memory trials, or appropriate measures of performance effects. The approach in the present study included: 1) discrete, paired-run, delayed alternation trials, which were analogous to delayed-matching-to-sample tests of working memory, 2) start, choice, and goal box latencies which provided direct measures of drug and age effects on performance, and 3) multivariate analyses to determine differential profiles of drug effects among different age groups.

22.17 AGE-ASSOCIATED DECREASE IN THE RATE OF CEREBROSPINAL FLUID UPTAKE OF Na IN THE FISCHER-344 RAT. Q.R. Smith, Y. Takasato* and S.I. Rapoport. Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore City Hospitals, Baltimore, MD. 21224.

The cerebrospinal fluid (CSF) has an important role in the regulation of the brain extracellular environment and in the removal of drugs and unwanted metabolites from the brain (CSF "sink" effect). During the first two months of postnatal life, the CSF formation rate increases approximately 5-10 fold. To determine whether significant changes occur with aging, we examined the rate of CSF formation in young (3 mo), mature (12 mo) and aged (24 and 34 mo) rats by measuring the rate of CSF uptake of Na-22.

Active transport of Na by the choroidal epithelium is the primary event in the secretion of CSF. Thus, the formation of CSF is closely related to the entry of Na into the CSF from plasma. This correlation was used to determine the relative CSF formation rate from the initial rate of CSF Na-22 uptake in the conscious rat. Briefly, Na-22 (15 μCi/kg) was injected into the femoral vein of partially-restrained Fischer-344 rats. Blood samples were collected from the femoral artery for 5 min, at which time the rat was killed by i.v. injection of sodium pentobarbital. Immediately thereafter, CSF was drawn by suction from the cisterna magna. Samples were analyzed for radiotracer content with a gamma counter. The inital rate of uptake of Na-22 into the CSF was expressed as a transfer constant which was calculated according to the equation:

$$\text{CSF transfer constant } (k) = C_{csf} / \int C_{plasma}\, dt.$$

In young (3 mo) rats, the CSF k for Na was $3.00 \pm 0.04 \times 10^{-4}$ sec^{-1}, which agrees with published values for the rat (Smith et al., J. Neurochem. 37:1117-1124, 1981. The CSF k of the 12 mo-old rat, $2.91 \pm 0.10 \times 10^{-4}$ sec^{-1}, was not significantly different from that of the 3 mo-old rat. In contrast, an age-associated decline in k was found in 24 and 34 mo-old rats. The CSF k decreased 6% to $2.73 \pm 0.09 \times 10^{-4}$ sec^{-1} between 12 and 24 mo ($P < 0.05$) and decreased by 18% to $2.38 \pm 0.14 \times 10^{-4}$ sec^{-1} between 12 and 34 mo ($P < 0.05$). These results suggest an age-related decrease in the rate of formation of CSF in the Fischer-344 rat. The fall in the rate of formation between 12 and 24 mo correlates well with the marked decrease in the extracellular volume of the rat brain between 3 and 24 mo (Bondareff and Narotzky, Science 176: 1135-1136, 1972). Together, the reduction in the rate of formation of CSF and the decrease in the volume of extracellular space would impede the removal of solutes of low permeability from the brain.

22.18 DECREASED DIAZEPAM-INDUCED TOLERANCE IN AGED C57BL/6J MALE MICE. P. Hicks, C. Rolsten*, T. Samorajski and J. Schoolar. Texas Research Institute of Mental Sciences, 1300 Moursund, Houston, Texas 77030.

It is widely reported that long-lived patients exhibit more side effects when given benzodiazepines than younger patients. The mechanism for the increased responsiveness is unknown, and requires further evaluation.

Male 12-, 18- and 28-month old C57BL/6J mice were used that had been purchased as retired breeders from Jackson laboratories (Bar Harbour) and aged in our housing facilities. The mice were housed 5/cage on a 12 hr. light/dark cycle (7am to 7pm) and fed Wayne Lablox and water ad libitum. Diazepam was administered intraperitoneally at weekly intervals. The first of six doses was 40mg/kg. Subsequent doses were 45mg/kg. Sleeping time and response latency time were measured for each mouse. Sleeping time was defined as the time period from loss of the righting reflex to recovery of the righting reflex. The time from drug administration to loss of the righting reflex was defined as the response latency time. Statistical comparisons were performed by X^2 analysis or the Duncan's New Multiple Range Test.

The initial dose of 40mg/kg of diazepam induced hypnosis in 50-70% of all mice tested (N=29,30 and 25 for the 12-, 18 and 28-month old mice, respectively). An attempt to produce a dose-response relationship by increasing the second weekly dose to 45mg/kg was frustrated by marked tolerance, the number of responding mice decreased to 20-55%. Therefore, no further attempts were made to increase the weekly diazepam dose. Maximum tolerance to the hypnosis-inducing effect of diazepam was not demonstrated in six doses given to the oldest age group. Maximum tolerance was reached on the fourth dose in both younger age groups. Hypnosis was induced in a greater number of old mice than in either younger age group at each dose. However, neither the response latency time nor the total time of hypnosis were affected by repeated doses of diazepam in any age group.

These results point out the marked degree of tolerance to the hypnosis-inducing effect of diazepam that can occur after a single dose. In addition, it is clear that the 28-month old mice are less able to develop this tolerance. This suggests that the mechanism for increased side effects to benzodiazepines in long-lived patients may involve a decreased ability to develop tolerance to these compounds. Further evaluation of biochemical and pharmcokinetic factors may be useful in characterizing the role of decreased tolerance in the increased responsiveness of the long-lived patients to benzodiazepines.

122.19 CHANGES IN RESPONSIVENESS OF THE INFERIOR COLLICULUS TO PURE TONE STIMULI IN AGING RATS USING 2-DEOXY-D[1-^{3}H] GLUCOSE. J. Coleman, W. J. Clerici and W. A. Cooper, Jr.*. Depts. of Psychology, Physiology and Communicative Disorders, University of South Carolina, Columbia, S.C. 29208.

Hearing loss is a characteristic feature observed in senescent subjects. Central auditory structures are also among those which show reduced glucose utilization in aging populations (Smith, Goochee, Rapoport & Sokoloff, 103: 351, 1980). We studied young and aged adult animals to determine changes in responsiveness to pure tone stimuli using the labelled 2-deoxyglucose method. Stimuli at 1, 2, 8, 40 or 50 kHz were monaurally presented to male albino rats 3-36 mo old following infusion of 2-deoxy-D[1-^{3}H] glucose through previously implanted intravenous catheters. Animals were sacrificed 45 min after infusion, perfused and frontal sections cut at 20 μm thickness. Sections were exposed to high contrast X-ray film and later stained with thionin. Computer image processing provided quantification of optical densities.

In 3 mo animals the central nucleus of the inferior colliculus showed discrete stimulus-induced isofrequency bands, particularly at 8 kHz and above. The location of the band was frequency-dependent with high frequencies represented ventromedially and low frequency bands observed dorsolaterally. Using the same frequency/intensity parameters as for 3 mo animals, stimulation in rats 27 mo or older produced abnormal patterns in the inferior colliculus at all frequencies tested. At 40 or 50 kHz stimulation discrete contralateral banding was absent in several cases and was replaced by diffuse, reduced activity instead. Other high frequency cases exhibited disrupted bands or even isolated spots of activity. Most lower frequency cases were characterized by a reduction or absence of banding activity in the inferior colliculus. Unstimulated ear-plugged aged rats showed lower metabolic activity in the colliculus than unstimulated young adults. Otoscopic examination revealed all animals had clean ear canals and normal-appearing tympanic membranes. Further, tympanometry indicated all animals had compliance peaks at ear-canal pressures which approximated atmospheric pressure. Maximum compliance was slightly higher for younger subjects. These results indicate that age-related differences were not attributable to external or middle ear changes but to sensorineural differences in responsiveness to acoustic stimuli. (Supported by NIH AG-1571.)

122.20 RESPONSE CHANGES OF INFERIOR COLLICULUS NEURONS IN AGING C57BL/6 MICE: GAINS AND LOSSES. J.F. Willott, Dept. of Psychology, Northern Illinois University, DeKalb, IL 60115.

As part of a study of aging and neural coding in the auditory CNS, extracellular responses of neurons in the central nucleus of the inferior colliculus (ICC) were studied in C57BL/6 mice of 3 age groups: 28-35 day-olds (Group I); 6-7 month-olds (Group II); 12-13 month-olds (Group III).

ICC neuronal responses clearly show a severe and progressive loss of high frequency sensitivity between 1 and 13 months of age. For instance, in Group III it is rare to find neurons responding to tones of more than 25 kHz, whereas in Group I, such neurons are common, with many upper ranges exceeding 50 kHz. This is consistent with the results of previous studies by D.O. Mikaelian, K.R. Henry, and their respective colleagues, who also showed that peripheral degenerative processes accompany these losses.

Neurons throughout the ICC show an age-related decrease in the maximum discharge rates evoked by tones, and neurons in older animals are less likely to respond to tones with sustained discharge patterns. On and on-burst response patterns predominate. Nevertheless, intensity function slopes obtained at neurons' best frequencies increase with age.

Analysis of response properties as a function of dorsoventral location of the neurons reveals that tonotopic organization is severely disrupted in older C57BL/6 mice. As high frequency sensitivity drops off, neurons in the more ventral regions of the ICC (normally most responsive to high frequencies) can no longer respond. However, many neurons in these areas appear to have undergone a change in their response areas. Rather than decreasing their frequency ranges with age (as might be expected with loss of high frequency sensitivity), their frequency ranges have expanded. This is due primarily to the extension of response areas into lower frequencies in neurons of the ventral half of the ICC. For instance, in rather ventral, high frequency areas, it is unusual to find neurons that respond to frequencies of less than 9-10 kHz. In mice of Groups II and III, the majority of response areas extend to frequencies well below 8 kHz in this region of the ICC.

Thus, despite age-related declines in sensitivity to high frequencies and robustness of discharge rates, there is also an increase in the volume of ICC that is capable of responding to low frequency sounds. This may represent a mechanism that compensates for the decline of peripheral sensitivity with age by increasing responsivity of the CNS to frequencies to which the system remains relatively sensitive.

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122.21 F-WAVES AS AN INDEX OF AGING

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The F-wave is due to small recurrent discharge of a few motor neurons produced by an antidromic volley in the motor fibers of the peripheral nerve. The value of determination of various parameters of F-waves, such as, latencies, amplitudes and conduction times have been recognized recently in the diagnosis of spinal roots and peripheral nerve disorders. The latencies, amplitudes and conduction times of the F-waves not only depends on the functional status of these structures, but also on the motor neurons, as it is considered to be a centrifugal discharge of the later. The electrophysiologic and histologic evidences suggest that motor units drop with age - a physiologic observation. If the F-waves determine the functional status of motor neurones, the change of various parameters of F-wave would also occur with aging process. To prove this hypothesis, the F-wave latencies, amplitudes and F/M ratios (M=compound muscle action potential) are determined in control population (A: 20-40 years, B: 40-60 years and C: 60 years and up) in the median and tibial nerves. The peripheral sensori-motor nerve conduction studies were normal in all. All these parameters were found to be significantly altered with advancing age, especially in Group C.

It is known that the underlying defect in the neuromuscular system during aging is secondary to progressive motoneurone dysfunction. The fall in the motor neurons and motor unit pool as a result of aging process would lead to axonal sprouting, thereby increase the motor unit territory of a neurone. This mechanism leads to change in the F-wave amplitudes and F/M ratios. These observations should always be considered, especially in the electrophysiologic evaluation of peripheral neuro-muscular function in aging population, otherwise may lead to misinterpretation, especially in the presence of normal peripheral nerve conduction studies.

123.1 CONTRIBUTION OF THE DENTATE NUCLEUS TO SET. J. Hore and T. Vilis. Department of Physiology, Univ. of Western Ontario, London, Ont., Canada, N6A 5C1.

Previously we demonstrated that, following a torque pulse perturbation which stretched biceps, a phasic EMG response(peak 70 ms) occurred in triceps which helped stop the return movement. Normally this antagonist response began before stretch of triceps, but during cooling of the dentate nucleus it followed stretch of triceps. A question that arose from this study was whether the 70 ms antagonist response resulted entirely from a stereotyped CNS signal based only on afferent feedback, or whether it was dependent on set i.e. a consequence of the monkey being able to predict the nature of the perturbation.

To examine this question we trained 5 Cebus monkeys to resist torque pulse perturbations (40 ms duration) and then, unexpectedly, introduced a torque step perturbation (for which a 70 ms antagonist response was inappropriate). In a different experiment we trained these monkeys to resist torque steps and then unexpectedly introduced a torque pulse. Four types of EMG responses were collected depending on what perturbation was expected (Set) and what perturbation was delivered (Get) (I: Set Pulse-Get Pulse, II: Set Pulse-Get Step, III: Set Step-Get Step, IV: Set Step-Get Pulse). Comparing EMG responses of I and IV we found that the M2 agonist response (peak 50 ms) was larger in IV, that a small later agonist (M3, 70 ms) was present in IV but not in I and that the normal 70 ms antagonist response in I was delayed in IV. Comparing the EMG responses of II and III we found that both 50 ms and 70 ms agonist responses were decreased in II and that a small 70 ms antagonist was frequently present in II but not in III. Since in each pair (I and IV, II and III), the perturbation and thus the initial afferent input was the same, any differences in the EMG response must be due to differences in set. The function of this set appears to be a) to increase the gain of the 50 ms agonist response when a step is expected and b) to direct a later 70 ms EMG response to the agonist when a step is expected and to the antagonist when a pulse is expected.

We also studied the EMG responses to pulses or steps during cooling of the dentate nucleus. We found that the antagonist EMG response to an expected pulse during cooling was qualitatively similar to the normal unexpected pulse response (IV) and that the expected agonist step response during cooling was similar to the normal unexpected step response (II). Thus the main effect of a lesion of the dentate nucleus was the elimination of set.

The main conclusion of this study is that the normal EMG responses to limb perturbations are dependent both on set and afferent feedback while those during cerebellar lesions are dependent strictly on afferent feedback.

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123.2 ELECTROPHYSIOLOGICAL STUDY OF THE CORTICONUCLEAR PROJECTION IN THE CAT CEREBELLUM. Q.X. Yu*, T.J. Ebner and J.R. Bloedel (SPON: C. Terzuolo). Departments of Neurosurgery & Physiology, University of Minnesota, Minneapolis, 55455

Anatomical and electrophysiological data support the concept of a sagittal organization of the corticonuclear projection of the cerebellar cortex. This study is the first in a series designed to examine the physiological properties of this pattern of organization based on the responses of Purkinje cells whose site of termination is demonstrated by their antidromic activation from the cerebellar nuclei. In 52 adult, decerebrate unanesthetized cats bipolar stimulating electrodes were stereotaxically positioned in the fastigial (FN), lateral vestibular (LVN) and interposed nucleus (IN). In 6 animals a systematic study of the antidromic field evoked on the surface of lobule V by stimulation in these three sites revealed a very reproducible border that demarcated the distribution of the fields evoked by the FN and IN stimuli. In this lobule the field responses to the LVN stimulus were comparatively small and scattered medial to this border. In 15 animals the distribution of the antidromic responses of extracellularly recorded, identified Purkinje cells in lobules Va-c to stimulating in the cerebellar nuclei was determined. Cells antidromically activated from the fastigial and interposed nuclei were separated by a discrete sagittal border located 2.1 ± .12 mm from the midline. Cells medial to this border projected predominantly to the FN with a few Purkinje cells projecting to the LVN. Neurons up to 2.5 mm lateral to this border projected solely to the IN. To begin to assess the functional differences of these corticonuclear zones the responses of 200 Purkinje cells to ipsilateral forepaw flexion was determined. Of 115 Purkinje cells projecting to the FN, 60 increased and 20 decreased their simple spike discharge in response to this peripheral input. Of 7 Purkinje cells projecting to the LVN only 1 was modulated by the forepaw stimulus. Cells projecting to IN were appreciably more responsive to the forepaw stimulus than those to the FN, with 68 of 85 being activated and 12 showing a reduction in simple spike activity. In addition the response amplitude of neurons projecting to IN were usually greater than those cells projecting to the FN. Thus Purkinje cells in both zones are responsive to this specific peripheral input. Their distribution was highly correlated with that of climbing fiber inputs activated by the same stimulus. Thus location of Purkinje neurons activated by mechanical stimulation of the forepaw may be more closely related to the zone of climbing fiber inputs responding to the stimulus than to the location of the identified corticonuclear zones. This work was supported by NIH Grants #RO1-NS 09447-10 and 1RO1-NS 18338-01.

123.3 GABAERGIC INNERVATION OF THE RABBIT INFERIOR OLIVE STUDIED BY GAD-IMMUNOCYTOCHEMISTRY. E. Mugnaini, N. H. Barmack and W. H. Oertel*. Biobehavioral Sciences, and Physiology Section of The Biological Sciences Group, The Univ. of Conn., Storrs, CT, and Dept. of Neurology, Technical Univ., Munich, FRG.

Since the inferior olive contains a basically uniform cell type, conventional histological techniques have not been useful for distinguishing functional subdivisions. Rather, the anatomical knowledge of these subdivisions has been based on the topography of the inferior olive and on its afferent and efferent projections. We have re-investigated the histology of the inferior olive of the rabbit at the light microscopic level using an immunohistochemical technique which reveals the distribution of glutamic acid decarboxylase (GAD), the GABA-synthesizing enzyme. Our aim was to determine if this histochemical technique could provide an improved description of functional subdivisions within the inferior olive. Rabbits were perfused transcardially with low and neutral pH aldehyde/salt solutions, which enhance axonal immunostaining and cell body immunoreactivity respectively. Vibratome sections were processed for the PAP procedure employing a sheep antiserum to rat brain GAD (Oertel et al., 1981). No GAD-positive cell bodies were found in the inferior olive. However, all subdivisions of the inferior olive contained a high density of GAD-positive boutons. Four areas of the inferior olive showed a particularly high staining density: 1) The beta nucleus of the medial accessory olive (MAO) stained the most densely of any of the subnuclei of the inferior olive. The boutons impinging on the beta nucleus had the largest diameter. 2) The dorsal medial cell column (dmcc) of the MAO received smaller boutons of lower density than the boutons of the beta nucleus. 3) The dorsal cap of the MAO, and 4) the caudal part of the dorsal accessory olive (DAO) received smaller boutons of lower staining density than the boutons of the dmcc or the beta nucleus. It is possible that these differences in bouton size reflect different origins of the parent axons. Three of the four areas which stain most densely for GAD are implicated in visual-vestibular functions of the cerebellum. In addition, the beta nucleus and the dmcc receive projections from the ipsilateral vestibular complex (Saint-Cyr and Courville, 1979). These histochemical data, combined with physiological data, suggest that this vestibular projection to the olivary nuclei may be functionally inhibitory. Attempts to obtain direct evidence for the extrinsic origins of GABAergic afferents to the inferior olive using physiologically guided injections of retrograde tracers in conjunction with GAD-immunocytochemistry are in progress. (Supported by USPHS grant 09904, USPHS grant EY04167 and DFG grant Oe951-2-1).

123.4 THE DISTRIBUTION OF SEROTONIN (5HT*) AND SUBSTANCE P (SP*) IN THE RAT INFERIOR OLIVE. G.A. Bishop and R.H. Ho. Dept. of Anat., Coll. of Med., Ohio State Univ., Columbus, OH 43210.

Available data on the localization of 5HT in the inferior olivary complex (IOC) of the rat is contradictory and the location of SP has not been described in detail. Thus, we have examined the distribution of 5HT and SP in the IOC of the rat using Sternberger's PAP technique. 5HT immunoreactive varicosities were found throughout the dorsal accessory olivary nucleus (DAO) with the greatest density localized in the lateral part of the nucleus. 5HT immunoreactive varicosities were also present throughout the medial accessory olivary nucleus (MAO). However, the most intense labeling was found at caudal levels of the nucleus. The principal olivary nucleus (PO), as well as the other olivary nuclei, exhibited little, if any, 5HT immunostaining. The distribution of SP varicosities was heavy throughout the entire rostro-caudal extent of the DAO; the majority of labeling was found lateral to the XIIth nerve. Numerous SP immunoreactive varicosities were found caudally in the most medial aspect of the MAO. At more rostral levels, the number of labeled SP elements decreased in the MAO. Finally, SP immunoreactivity was seen in the medial extremes of both the dorsal and ventral lamella of the PO and in the dorsomedial cell column. Taken together, these data show an overlap in 5HT and SP varicosities primarily in the lateral DAO. However, there are areas within the IOC which contain only one of the two putative neurotransmitters. A previous study using the technique of retrograde transport of horseradish peroxidase (Bishop and King, Soc. for Neurosci. Abst., 7: 26, 1981) has shown that neurons in the n. raphe obscurus, n. raphe pallidus and n. gigantocellularis are sources of afferents to the IOC of the rat. A recent immunofluorescent study (Johansson et al., Neurosci., 6: 1867, 1981) demonstrated 5HT and SP in the somata of neurons located in these nuclei. In fact in some cases, both substances were localized in the same neuron. Double labeling experiments must be carried out to determine whether the cells described in the retrograde tracing study are the source of 5HT and SP observed in the IOC. (Supported by NIH NS-18028, NIH NS-10165, and the Bremer Foundation, The Ohio State University College of Medicine.)

*A substance's immunoreactivity is referred to by its name.

123.5 THE INFERIOR OLIVARY NUCLEUS OF THE RAT: A TECTO-OLIVO-MIDVERMAL PATHWAY. S. A. Azizi, R. A. Burne and D. J. Woodward. Dept. of Cell Biol., The Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

This study was undertaken: 1) to visualize the three-dimensional structure of the inferior olivary complex by computer assisted serial reconstruction of histological sections; 2) to determine precise olivo-midvermal projections and 3) demonstrate tectal projections to the areas of the inferior olive which in turn project to the midvermis. The techniques of retrograde transport of HRP and orthograde transport of 3H-amino acids were employed.

Sixty-eight Long-Evans hooded rats received single horseradish peroxidase (HRP) injections (.01 - .02 µl, 10 - 20% in saline) in midline and lateral areas of individual midvermal sublobules VIa - IXc. The locations of retrogradely labeled cells were mapped relative to a three-dimensional biological coordinate system maintained by a computer linked to a light microscope. In general the cerebellar posterior vermal zones receive climbing fiber projections from the caudal area of the inferior olivary complex. A distinct topographical organization within the olivo-midvermal projection system was noted in which injections on the midline of lobules VI - IX resulted in labeling of neurons in far caudal and lateral regions of the medial accessory olive (MAO). Injections (500 µ width) in a slightly lateral to the midline zone of the same lobules resulted in the labeling of a column of cells in the ventro-medial aspect of the MAO and nucleus beta. These data based on retrograde studies demonstrate for the first time that discrete cell groups within the MAO project to different adjacent sagittal zones in the cerebellar posterior vermis.

3H-Leucine was injected into the superior colliculus of 15 animals. The subsequent anterograde transport and terminal labeling over the specific regions of ipsilateral and contralateral MAO was visualized. Autoradiographic silver grains were primarily deposited in a bilateral manner over the ventromedial regions of the medial accessory olive and the nucleus beta. These observations indicate that the specific MAO region which receives visual information from the tectum also projects to sagittal strips located off the midline in the midvermal zone of the cerebellum.

In conclusion, by employing computer assisted reconstruction it has been demonstrated that there is a precise correspondence between the tectal efferent terminals and the olivary cells of origin projecting to sagittal zones of the midvermis. (Supported by NIDA 2338, AA-0390 and the Biol. Hum. Foundation).

123.6 ANALYSIS OF CAUDAL BRAINSTEM AND SPINAL CORD PROJECTIONS TO THE PONTINE GRAY IN RATS. R.S. Swenson*, R.J. Kosinski and A.J. Castro, Depts. of Anat., National College of Chiropractic, Lombard, IL 60148 and Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153.

Pontine afferents from the caudal brainstem and spinal cord have been less extensively studied than cortical, tectal and cerebellar inputs. This study was undertaken to characterize these projections using the Fink-Heimer stain and autoradiography. Under pentobarbital anesthesia (40mg/kg.) Long-Evans black-hooded rats sustained lesions of the spinal cord (at mid-thoracic or high cervical levels), the nucleus gracilis or the nucleus cuneatus, while other animals received .01 to .02µl injections of tritiated leucine (50µCi/ul) into the dorsal column nuclei or the spinal trigeminal nucleus. In order to define the somatotopy of dorsal column nuclear projections, a lesion was made in the right nucleus gracilis and the left nucleus cuneatus in four animals, and three other animals received a right gracilis lesion combined with a right cuneatus injection. At 5-10 days postoperative, animals were overdosed with pentobarbital and perfused with saline followed by 10% formalin. Brains were removed, sectioned frozen at 40µ, and stained by the Fink-Heimer protocol or processed for autoradiography.

The dorsal column nuclei projected to two regions of the contralateral caudal pontine gray (PG), one in the medial pontine subdivision and the other overlapping the lateral and ventral subdivisions. Cuneatus fibers terminated more rostrally and, within the medial pontine subdivision, more dorsally and laterally then those from gracilis. Spinal trigeminal projections terminated most heavily within the contralateral PG at mid-pontine levels, in two fields each being rostral and dorsal to the regions receiving cuneatus inputs. Both high cervical and mid-thoracic spinal cord lesions produced a similar pattern of degenerating fibers in the ipsilateral PG, which overlapped with gracilis inputs.

This study has identified a somatotopy in caudal brainstem and spinal cord projections to the PG. Generally, caudal portions of the body were found to project most caudally in the pons while the forelimb and head were found to be represented at progressively more rostral levels. Additionally, the terminal field in the medial pontine subdivision manifested a ventromedial to dorsolateral somatotopy for hindlimb to head, respectively. Mid-thoracic and upper cervical cord lesions revealed no somatotopic distribution of spinopontine projections. One possible explanation for this finding may be that the upper cervical cord gives rise to few spinopontine fibers.

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123.7 TOPOGRAPHIC ANALYSIS OF DORSAL COLUMN NUCLEAR AND MOTOR CORTICAL PROJECTIONS TO THE BASILAR PONTINE GRAY IN RATS. Ross J. Kosinski, G. Kartje-Tillotson, and Anthony J. Castro, Dept. of Anatomy, Loyola Univ. Stritch Sch. of Med., Maywood, Illinois 60153.

The present study was undertaken to determine the somatotopic distribution of dorsal column nuclear projections to the pontine gray and to examine their distribution patterns in relation to motor cortical inputs. Combining autoradiographic and degeneration methods within individual animals facilitated this analysis.

Stereotaxic injections of tritiated leucine (50µCi/µl) and lesions by aspiration were made in animals under ketamine hydrochloride anesthesia. Two groups of animals were used. The first group received 0.01-0.02µl injections into the left nucleus cuneatus combined with lesions of the left nucleus gracilis, and the second group received 0.3µl injections into the left hindlimb motor cortical area as determined by intracortical microstimulation (0.25msec. pulses, 350hz, 300msec. train, 10-100 microamps) combined with lesions of the right nucleus gracilis.

Both dorsal column nuclei demonstrated a substantial projection within the caudal half of the contralateral medial pontine nucleus. Within this nucleus fibers from cuneatus distributed rostral to fibers from gracilis, and additionally the cuneatus distribution was dorsolateral to the gracilis pattern. Less dense and less segregated labelling was found in the lateral and ventral pontine nuclei. Analysis of hindlimb sensorimotor corticopontine projections revealed a topographic distribution which overlapped with afferents from nucleus gracilis. As cortical mapping studies have demonstrated sensorimotor overlap for hindlimb but not forelimb cortical regions, further studies are in progress to examine the topographic relationships of forelimb corticopontine fibers to pontine afferents from nucleus cuneatus. Preliminary data indicates that motor forelimb does not overlap with pontine afferents from nucleus cuneatus.

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123.8 ROLE OF DENDRITIC ELECTRORESPONSIVENESS IN NEURONAL INTEGRATION: IN VITRO STUDY OF MAMMALIAN PURKINJE CELLS. M. Sugimori and R. Llinas. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York 10016.

The demonstration of electrical excitability in dendrites has raised important questions regarding the integrative properties of central neurons. In the Purkinje cell, dendrites are capable of dendritic electroresponsiveness (Llinas & Nicholson, J. Neurophysiol. 34:532, 1971). The electroresponsiveness which is Ca-dependent (Llinas & Hess: Proc. Natl. Acad. Sci. 73:2520, 1976 and Llinas & Sugimori: J. Physiol. 305:171, 1980) manifests itself in (a) generation of prolonged all-or-none depolarizations having a plateau-like appearance and (b) full action potentials which generally occur in bursts. These two forms of Ca-electroresponsiveness can be obtained by either direct stimulation of the soma and/or dendrite of this cell, by the activation of synaptic inputs (parallel fiber or climbing fiber) and by the iontophoretic application of glutamic acid (Sugimori & Llinas, Soc. Neurosci. Abst. 7:76, 1981). The present study was designed to determine the contribution of passive and active dendritic properties to synaptic activation. Glutamic acid was applied at different levels in the dendrite utilizing simultaneously two iontophoretic electrodes and one intrasomatic or intradendritic recording electrode. The potential obtained at these two levels by iontophoretic injection was compared with the potentials obtained following the blockage of dendritic voltage-dependent Ca conductance change. The results indicate that the synaptic potentials observed at the somatic level, which often appear to have no active components, due to their smooth time course, have sizeable Ca electroresponsive components. This subthreshold electroresponsiveness is often related to the plateau-type dendritic response but, in addition, when the depolarization is large enough, actual Ca spikes may be observed. Both type responses are blocked at dendritic level by iontophoretic application of GABA. We conclude that the conductance of synaptic potentials to the soma can be boosted, to varying degrees, by the Ca conductance properties of the dendrite. More important, because this boosting property may not seriously alter the smoothness of the synaptic potential observed at somatic level, their presence may have passed unnoticed in other central neurons. Supported by USPHS grant NS13742 from NINCDS.

123.9 THE SUPERNORMAL PERIOD OF THE PARALLEL FIBERS: EFFECTS OF $[Ca^{2+}]_o$ AND $[K^+]_o$. R.C. Malenka and J.D. Kocsis. Dept. of Neurology, Stanford Sch. of Med. and V.A. Medical Ctr., Palo Alto, CA. 94304.

The nonmyelinated parallel fibers (Pfs) of the cerebellar cortex exhibit a pronounced supernormal period (SNP) following a single conditioning volley as evidenced by a decrease in the latency of the Pf volley. In this study, we compare the effects of changes in extracellular calcium concentration ($[Ca^{2+}]_o$) on the SNP of the Pfs with changes in extracellular potassium concentration ($[K^+]_o$). Rat cerebellar Pfs were continuously superfused with normal Ringer solution (NS) or with NS containing varying concentrations of K^+ ($[K^+]_s$; 5-30 mM) or Ca^{2+} ($[Ca^{2+}]_s$; 0-6 mM). Microstimulation of the Pfs was employed and the "on-beam" Pf field potential recorded within 80 µm of the cerebellar surface using a Ca^{2+}-sensitive microelectrode.

Recovery properties were studied by monitoring control (unconditioned) and test (conditioned at 15 msec) response latencies while experimental solutions equilibrated in the superfusion pool. Changing the superfusate from NS (2 mM $[Ca^{2+}]_s$) to a solution containing no Ca^{2+} caused equal decreases in the control and test Pf volley latencies. Increasing $[Ca^{2+}]_o$ increased both control and test latencies. Both control and test Pf volley latencies were shown to be linearly related to $[Ca^{2+}]_o$. Changes in $[Ca^{2+}]_o$ therefore had little effect on the activity-dependent relative increase in Pf excitability observed following conditioning stimulation, i.e. the SNP.

In contrast to the effects of $[Ca^{2+}]_o$, $[K^+]_o$ did affect the SNP. Increasing $[K^+]_s$ (from 3 to 7-12 mM) elicited a decrease in the control Pf volley latency but had no effect on the test latency. This resulted in a reduction of the latency shift elicited by a conditioning stimulus. No change in $[Ca^{2+}]_o$ was recorded during these experiments or when $[K^+]_s$ was increased to 30 mM. When $[Ca^{2+}]_s$ was decreased and $[K^+]_s$ increased (to 7 mM) simultaneously, the control Pf volley latency decreased further than when each ion was altered separately. The test latency remained unchanged. Therefore, under these conditions, there was no Pf volley latency change following conditioning stimulation.

These results are consistent with the hypothesis that activity-dependent changes in $[K^+]_o$ and $[Ca^{2+}]_o$ may, in part, be responsible for Pf supernormality. Increases in $[K^+]_o$ most likely act via membrane depolarization while the effects of changes in $[Ca^{2+}]_o$ are consistent with the postulated biophysical action of Ca^{2+} on the axon membrane. (Supported by the Veterans Administration and the National Multiple Sclerosis Society.)

123.10 SYNAPTOGENESIS IN THE RAT BASILAR PONTINE NUCLEI. G.A. Mihailoff Dept. of Cell Biology, U.Tex. Hlth. Sci. Ctr., Dallas, TX 75235.

As a part of ongoing studies concerning the extrinsic connections, cytology, and synaptic organization of the basilar pontine nuclei (BPN) in adult and neonatal rats, we report here several observations regarding synaptogenesis in the BPN during the first 20 days of postnatal life (birth=day 0).

Synapse formation appears to begin during an early postnatal period which extends from day 5 to day 8. The accumulation of morphologically mature synapses is gradual during this period and is characterized initially by the appearance of small boutons (< 0.6 µm) which contain clear, round synaptic vesicles and form asymmetric contacts with small dendritic profiles. By postnatal days 7 and 8, the neuropil has a more organized appearance than earlier periods while the number of presynaptic profiles has increased considerably and their morphology become quite heterogenous. In several cases, lesions were placed in sensorimotor cortex during the postnatal period of day 2 to day 4 and the animals permitted to survive until day 6, 7 or 8 whereupon the animals were sacrificed for electron microscopic observation. The size and appearance of degenerating boutons suggested that corticopontine synapses were among the first to attain morphological maturity.

A second phase in synapse maturation occurs during the day 8-14 period. Two prominent features of this phase are (1) the appearance of all categories of presynaptic profiles observed in the BPN of adult rats, although some varieties are quite sparse in number, and (2) a considerable increase in the number of synaptic contacts formed with structures that are readily identifiable as dendritic spines or protrusions.

These observations concerning BPN synaptogenesis correlate with two previous studies, one concerning the development of the corticopontine system, the other describing the morphogenesis of BPN neurons. First, autoradiographic studies indicate that although corticopontine projection fields are present in the early postnatal period (days 1-8), the adult configuration of such fields is not apparent until day 16. This implies that a process of constriction or maturation occurs within the day 8-16 period which in fact appears to be reflective of the synaptogenic events taking place in that time frame. Secondly, in Golgi studies which described the morphogenesis of pontine neurons, the major elaboration of dendritic spines was reported to occur within the day 10-12 period, while the maturation of the dendritic tree was completed by day 16, time periods which also appear to coincide with the present ultrastructural observations regarding synapse formation in the BPN.

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123.11 AN ELECTRON MICROSCOPIC STUDY OF THE DEVELOPMENT OF THE PURKINJE CELL IN THE CEREBELLAR CORTEX OF THE OPOSSUM. L.C. Laxson and J.S. King. Dept. of Anat., Coll. of Med., Ohio State Univ., Columbus, OH 43210.

The development of the Purkinje cell (Pk) in the cerebellar cortex of the opossum was analyzed using Golgi preparations and electron microscopy. The maturation process of the Pk cell will be described in five stages: the immature stage, the perisomatic dendrite stage, the perisomatic spine stage, the main dendrite stage and the adult stage. These stages were first defined by Hendelman and Aggerwal (J.C.N., 193: 1063, 1980).

Pk cell development as observed in electron micrographs is characterized by distinct synaptic relationships and cytological features in each of the five stages. During the first or immature stage (PN 19-32), the Pk cells have long apical processes which may be involved in the early migration of these neurons. Few boutons make synaptic contacts with the Pk cells during the early part of this first stage; synapses are more frequent by the end of the immature stage. The second or perisomatic dendrite stage (PN 33-44) is characterized by numerous somatic processes which have the cytological characteristics of dendrites. All synaptic contacts present during this stage form asymmetric contacts and contain round vesicles; however, the shape of the terminal and their postsynaptic location were used to tentatively distinguish different types of terminals. During the third or perisomatic spine stage (PN 45-60) the Pk cell has numerous somatic spines and the dendritic tree is achieving its mature form. Most synaptic terminals observed during this period have asymmetric junctions and contain spherical vesicles; both the perisomatic spines and the dendritic shafts and spines are the postsynaptic sites of these terminals. Another type of bouton has pleomorphic vesicles and forms indistinct symmetric junctions directly on the cell body. The fourth stage, the main dendrite stage (PN 61-75), is characterized by the loss of the perisomatic spines. Terminals on the soma contain pleomorphic vesicles and form symmetric synapses. The thorns of the primary and secondary dendrites and the more distal dendritic spines are postsynaptic to boutons with spherical vesicles and asymmetric junctions. The final stage or the mature stage (PN 76-adult) is characterized by the appearance of adult synaptic relationships.

These data suggest that although olivary axons are present in the cerebellum by PN 14 (Bishop, personal communication), synaptogenesis does not ensue until the latter part of the immature stage (PN 26-32). During the subsequent stages of Pk cell development, synaptic formation and remodeling proceed until the adult cytology and synaptology are achieved at about PN 75-80. (Supported by NS-08798.)

123.12 CONNECTIONS OF THE CAT RED NUCLEUS. F.R. Robinson*, J.C. Houk and A.R. Gibson. Northwestern Univ. Med. Sch., Chicago, IL. 60611.

Slight modifications of the Mesulam HRP procedure coupled with the use of HRP labeled lectins (Gonatas, et al., J. Histochem. Cytochem., 27: 728-734, 1979) produce an extremely sensitive, reliable and artifact free method for simultaneously tracing anterograde and retrograde pathways in the nervous system. We have used lectin transport to make a detailed study of the connections of the cat red nucleus (RN) with special emphasis on the magnocellular or spinal projecting area of the nucleus (RNm). Dense afferent termination is seen in RNm following injection into either the anterior or the posterior interposed nucleus. Only coursing fibers can be seen in RNm after injection of the lateral nucleus. The bulk of these fibers terminate in the thalamus and portions of the mesencephalon other than RN. The most anterior portion of the anterior interpositus, which is comprised of a distinct subnucleus of smaller cells, projects to a dorsal-medial portion of RNm which seems to correspond to the forelimb area of the RNm. A larger celled caudal portion of the anterior interpositus projects to the ventral-lateral portion of the RNm which corresponds to the hindlimb area of the nucleus. Medial portions of the posterior interpositus project in a shell that encases the RNm, more lateral regions of the posterior interpositus do not project to RNm. No dense projection to the parvocellular portion of the RN is seen following injection of either the lateral or interposed nuclei. Motor cortical projections are quite sparse and scattered in RNm with a heavier projection to parvocellular RN.

The heaviest projections from RNm are to the intermediate regions of spinal gray and to the lateral reticular nucleus. A few fibers in the cervical enlargement appear to terminate within motor neurons. Diffuse projections to many brainstem areas are also seen. The parvocellular portion of RN consists of small cells rostral to and separate from the spinal projection areas of RN. These cells terminate in the principal nucleus of the inferior olive. The detailed anatomical specificity between the interposed nuclei and the RN will be a valuable guide to understanding the functional organization of the cerebellum in the control of motor performance.

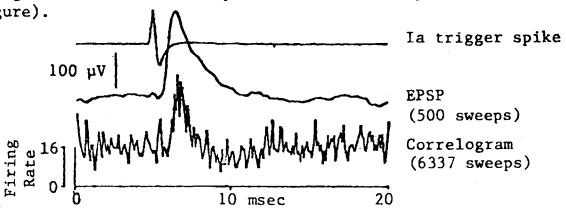
124.1 RELATIONSHIP BETWEEN MUSCLE TOPOGRAPHY AND TENDON ORGAN-MOTOR UNIT INTERACTIONS IN CAT TIBIALIS POSTERIOR. Connie Osborn and Marc D. Binder. Dept. Physiol. & Biophys., Univ. of Washington Sch. of Med., Seattle, WA 98195.

Tendon organs (TOs) are excited by an average of 10 motor units in a muscle, presumably those motor units of which 1 or 2 muscle fibers are directly attached to the receptor (Houk et al., Prog. in Clin. Neurophys. 8: 33, 1980). In medial gastrocnemius the motor units exciting a TO tend to be concentrated in a compartment in which the receptor itself is located (Cameron et al., J. Neurophys. 46: 32, 1981). One implication of this compartmentalization is that pairs of adjacent TOs would more often be excited by the same motor unit than would pairs of more distant TOs. In 5 experiments we isolated from the dorsal roots 3-4 (19 total) afferent fibers innervating TOs in tibialis posterior (TP). The location of each TO was approximated in the muscle by mechanical probing. Ventral roots L6 and L7 were divided to isolate from 25-65% of the total number of motor axons in TP (Boyd and Davey, Comp. of Periph. Nerves, 1968). Each motor axon was tetanized, and the tension developed and the activity of the isolated TO afferent fibers were recorded. Individual TOs were excited by from 2-15 motor units (av. 7.8±3.3); the 5 TOs with spontaneous activity were unloaded by 8-21 motor units (av. 13.2±4.9). Motor units exciting TOs produced tetanic tensions of from 2-220 g, the same range of tensions for the motor unit population as a whole, but exciting motor units tended to have higher tetanic tensions as a group than did non-exciting motor units ($P<.002$). Pairs of TOs shared from 0-6 exciting motor units. 5 of 27 pairs shared more than 3 motor units, a number significantly greater than that predicted from a hypothesis of random selection of completely intermixed motor units ($P<.001$). TP is longitudinally bisected by an intramuscular tendon, and the nerve bifurcates before entering the muscle, but this division of the muscle and nerve did not contribute to the distribution of shared motor units by TO pairs: 10 pairs of TOs which were located on the same side of the tendon shared the same number of motor units (2.0±1.8) as 17 pairs located on opposite sides (1.8±1.8). However, our data show that pairs of TOs located very close together in the muscle share significantly more motor units (4.5±1.7) than do TOs located at proximal and distal extremes of the muscle (0.7±.95).

(Supported by NIH grants NS 15404, NS 00345 and GM 07108.)

124.2 EFFECTS OF SINGLE FIBER IA-EPSPS ON FIRING PROBABILITY OF α-MOTONEURONS. T.C. Cope, M. Matsumura, and E.E. Fetz. Dept. of Physiol. & Biophys., Univ. of Washington, Seattle 98195.

The effects of single fiber Ia-EPSPs on firing probability of triceps surae motoneurons (MNs) were documented in barbiturate-anesthetized cats. Afferent spikes were evoked by steady muscle stretch and recorded from single Ia fibers in dorsal root filaments. Spikes from Ia fibers were used to trigger averages of intracellular records of MN membrane potential with the MN at rest to reveal the shape and amplitude of single fiber Ia-EPSPs. The same MNs were then induced to fire rhythmically (≤ 30 pps) by intracellular current injection in order to compute cross-correlograms between the Ia spikes and MN action potentials (see figure).



Data were obtained for 20 Ia-MN connections for which cross-correlograms included at least 2000 trigger spikes. At 13 of these connections, statistically significant correlogram peaks could be resolved. The mean increase in firing rate during the peaks ranged from 29-138% above baseline firing rate. Peaks had a mean onset of 0.3±0.1 ms (SEM) after EPSP onset and a mean duration of 2.5±0.1 ms (SEM). At these 13 connections the amplitudes of associated EPSPs ranged from 86-310 μV. At the remaining 7 connections no peaks could be identified in correlograms generated from 2000-7000 trigger spikes; the associated EPSPs had amplitudes of 25-86 μV. For all 20 connections the mean increase in firing rate during the correlogram peak (including cases for which no peak was present) was positively correlated with EPSP amplitude ($r=0.89$; $p< 0.001$). EPSPs averaged on the trajectory of MN membrane potential during repetitive firing just prior to the action potential often differed in amplitude compared to EPSPs averaged with the MN at rest, and as a group were slightly better correlated with the increase in firing probability. These findings indicate that under the conditions of these experiments, single fiber EPSPs ≥ 86μV significantly increase the firing probability of MNs in proportion to their size; EPSPs smaller than about 86 μV fail to produce detectable changes in firing probability. (Supported by NIH grants NS 12542 and NS 07097).

124.3 RESPONSES OF HINDLIMB EXTENSOR MUSCLE SPINDLES TO HEAD ROTATION IN DECEREBRATE CATS. R.Boyle* and O.Pompeiano. Ist.Fisiol.Umana, Catt. I,Univ.Pisa,56100 Pisa,Italy.

In previous investigations we studied the responses of Deiters' neurons,identified antidromically to project to lumbosacral spinal segments,to sinusoidal stimulation of vestibular and neck (joint and/or muscle) receptors (see J.Neurophysiol.45:852-868,1981). We have continued our analysis of vestibulospinal relations by examining the response of muscle spindle afferents (primary and secondary endings) of the isometrically extended (6-8 mm) gastrocnemius-soleus muscle to head rotation about the longitudinal axis in precollicular decerebrate cats.

After acute bilateral deafferentation of C_1-C_3 to eliminate possible influences arising from neck receptors, head rotation (at 0.026 Hz, ±15°) induced a weak periodic rate modulation in only 15.8% (6/38 units) of the tested spindles; maximum discharge increase" lagged peak of the ipsilateral side-down head displacement by -432±47.0°, SD (α-responses) and average response gain was 0.18±0.12 imp.sec.deg^{-1} (mean firing rate 189±28 imp.sec^{-1}). Across the frequency range between 0.008 to 0.325 Hz (±15°) response gain remained stable; phase angle of response, however, showed some variability, ranging from a phase advance relative to head position (in most spindles) to an increase in phase lag. In a second group of 38 spindle receptors head rotation following acute bilateral labyrinthectomy, thereby stimulating presumed neck receptors, failed to influence spindle firing rate in a reliable manner. In a third group of experiments in which eighth nerves and cervical dorsal roots were left intact, head rotation induced a response in 15.6% (7/45 units) of the tested spindles similar to that observed after neck deafferentation.

The results indicate that vestibular volleys, elicited by sinusoidal head rotation, can reach and influence fusimotor fibers supplying extensor hindlimb muscles, as inferred from the observed modulation in spindle discharge from the gastrocnemius-soleus muscle under isometric conditions. The difference in strength of response observed between Deiters' neurons and extensor muscle spindles to individual or combined stimulation of vestibular and neck receptors suggests that a considerable amount of processing-probably involving structures other than Deiters nucleus-occurs between labyrinth and neck inputs and fusimotor output to hindlimb extensors.

124.4 WHAT DO SPIKE-EVOKING LENGTH TRANSIENTS TELL US ABOUT MUSCLE RECEPTORS? W. Koehler*, R.M. Reinking*, R. Enoka, T.M. Hamm and D.G. Stuart (SPON: Z. Hasan). Dept. of Physiol., Univ. of Arizona Health Sci. Ctr., Tucson 85724.

Random-length stimuli were used to characterize muscle receptor properties in chloralose-urethan anesthetized cats. The medial gastrocnemius muscle was connected to a servo-puller system activated by a pseudorandom generator. The spike trains of functionally isolated muscle afferents were recorded at the dorsal root level simultaneous to length and force signals from the test muscle. "Spike-evoking length transients" (SELTs) were generated by use of each spike train as the trigger input to a signal averager whose input signal was the length signal. The resultant average provides an estimate of the mean length change pre- and succeeding the spikes. Our results are based on the SELTs of 11 spindle afferents (7 Ia, 4 spII) and 11 tendon organ (Ib) afferents. SELTs were obtained at 25 different combinations of stimulus amplitudes (0 to 5-100 μm) and bandwidth (0 to 5-100 Hz).

It has been previously shown that there is no striking difference in the SELT profile of different afferent types. The present work extends on this observation by: 1) analyzing the underlying theoretical reasons for the initial observation; 2) correlating SELT profiles with known receptor properties as revealed by ramp and step-like stretches; and, 3) demonstrating the effect on SELT profiles of spontaneous spikes in the trigger train.

Our results show that the SELT profile is determined primarily by the white-noise characteristics of the stimulus and is interpretable on the basis of the deviation from ideal white noise revealed in the autocorrelogram-estimate of the length signal. Nonetheless, quantitative differences in receptor sensitivity (particularly Ia vs. spII and Ib) are brought out by comparing SELTs for different "intensities" of length stimulation. The relation between negative (shortening) and positive (lengthening) components of each SELT profile is shown to reflect each receptor's relative sensitivity to absolute muscle length and its rate of change. Finally, the problem of the contribution of "spontaneous" spike triggers to each average is shown to be approachable by use of the standard deviation of the SELT profile at the presumed moment of spike generation.

In conclusion, this analysis reaffirms current understanding of the static and dynamic properties of muscle receptors as revealed by use of other forms of muscle perturbation, and further provides insight into the "ground rules" required for the interpretation of the SELT, particularly under the conditions of random-length stimulation and by consideration of the autocorrelative properties of the test spike trains. (Supported in part by the DAAD (West Germany) and USPHS grants NS 07888, HL 07249 and RR 05675).

124.5 SINGLE UNIT ANALYSIS OF THE ADEQUATE STIMULUS TO SMALL MYELINATED AFFERENT FIBRES SUPPLYING DORSAL MUSCLES OF THE CAT NECK.
V.C. Abrahams, B. Lynn*†, and F.J.R. Richmond, Department of Physiology, Queen's University, Kingston, Ontario, Canada. K7L 3N6

Afferent fibre spectra of nerve bundles entering biventer cervicis and complexus show that these bundles contain large numbers of 3-6 μ myelinated fibres. Single fibre electrophysiological analysis has now shown that a significant percentage of small myelinated axons take origin in cutaneous receptors whose axons have an intramuscular course. However, a further 228 afferent fibres which had their receptors in dorsal neck muscle were also dissected and examined electrophysiologically. Approximately 20% of these fibres had conduction velocities between 5 and 30 m/sec and were thus considered Group III fibres. These axons supplied receptors with physiological properties different from those of muscle spindles or Golgi tendon organs. When the response of these units to local pressure, local stretch and contraction was examined, the majority of these slowly conducting units were found to be high threshold mechanoreceptors which were activated by stretch, by local pressure, or by both stretch and pressure. The stimulus strength required for activation of small fibres was much greater than stimuli required to excite spindles and Golgi tendon organs.

In general, the high threshold units had a small focus of maximum sensitivity which was close to a muscle border or a tendinous inscription. Few units could be activated by muscle contraction. Intra-arterial injections of bradykinin into the dorsal neck muscles through the brachial artery did not prove adequate to excite the small myelinated fibres even though the same dose was able to activate Group IV fibres (c.v.< 2 m/sec) present in the same filament. Similarly frequency of firing in small myelinated fibres was not modified by the intramuscular injection of bradykinin in close proximity to the receptor. In contrast, the few receptors which were exposed to the intramuscular injections of 6% sodium chloride discharged for several minutes following that injection.

It is concluded that the Group III muscle afferent fibres in the dorsal rami of the neck probably belong to a class of high threshold mechanoreceptors. There is no clear evidence to suggest that these receptors are primarily concerned with the perception of pain.

Supported by the Medical Research Council of Canada.
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124.6 PRIMARY AFFERENT PROJECTIONS FROM DEEP NECK MUSCLES IN THE CAT: AN ANATOMICAL STUDY USING TRANSGANGLIONIC TRANSPORT OF HORSERADISH PEROXIDASE.
D. A. Bakker*, F. J. R. Richmond and V. C. Abrahams (SPON: H. Dinsdale). Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Major postural deficits are well-known to follow the disturbance of sensory systems in neck muscles. Effects on posture and tonic neck reflexes are especially marked when the locus of damage includes the afferent supply from paravertebral tissues. The paravertebral musculature is now known to contain an unusually dense network of complex muscle receptors, but the central connections of these receptors are not well understood. The transganglionic transport of horseradish peroxidase (HRP) has therefore been used to map the projections of afferent fibres from paravertebral muscles around C1-C2 including rectus capitis posterior muscles and obliquus inferior. Muscle nerves were sectioned at their points of muscle entry and exposed to HRP solution for 4 hr. Tissue was processed with conventional methods using TMB.

Labelled axons entered the dorsal roots and turned to course rostrocaudally in the dorsolateral quadrant of the dorsal columns. Many labelled axons also ran ventrally along dorsal horn of C1-C3 and ramified extensively in the medial part of the intermediate grey matter. Reaction product was distributed throughout the central cervical nucleus and in adjacent dorsomedial and ventral regions. Labelled fibres also entered the ventral horn but their distribution patterns were obscured by the simultaneous retrograde filling of motoneurone dendrites.

Labelled fibres ascended into the medulla in a bundle between the cuneate nucleus and spinal nucleus of V. At medullary levels, three consistent regions of HRP deposition could be identified. Deposition was present in a column throughout the intermediate nucleus of Cajal. This deposit continued rostrally from that associated with the central cervical nucleus at C1 spinal levels. A second restricted zone of sparse labelling was located in the ventrolateral part of the cuneate nucleus. A third zone of dense termination extended throughout ventromedial and rostral regions of the external cuneate nucleus. Primary afferent fibres from deep neck muscles thus have a restricted pattern of projection into the spinal cord and medulla. Many afferent fibres appear to terminate in regions known to supply afferent projections to the cerebellum.

(Supported by MRC of Canada).

124.7 POSITION AND VELOCITY SENSITIVITY OF JAW MUSCLE SPINDLE AFFERENTS DURING CONTROLLED ISOTONIC JAW MOVEMENTS IN THE MONKEY.
D.V. Finocchio, C.R. Larson*, A. Smith* and E.S. Luschei*. Dept. of Physiol. and Biophysics, Univ. of Wash., Seattle, WA 98195.

In order to study, in a quantitative fashion, the relationship between the firing rate of jaw muscle spindle afferents and the position and movement velocity of the mandible, we trained monkeys to "track" a target by controlling the position of a lever arm with their jaws. Force necessary to control the arm was constant at all positions, and could be varied by adding weights. In one condition, the animal held the mandible steadily at the specified position for periods of about 2 sec. In the other condition, the monkey tracked a "triangle" waveform that covered the entire range of movement once every 10 sec. This condition produced periods of slow, controlled jaw movement. The activity of cells in the Mes. Nuc. of the Vth nerve (cell bodies of jaw muscle spindle afferents) was recorded during these behaviors. Twenty-one secondary and 26 primary afferents (characterized on the basis of their behavior) were studied in detail.

All secondary endings exhibited a consistent, linear relationship between firing rate and static jaw position. The slope of the linear regression was typically between 4 and 6 spikes/sec/mm of incisal opening. Correlation coefficients for such regressions were typically between 0.8 and 0.9. Slow movements did not alter the position sensitivity of these cells, nor did substantially increasing the load. Secondary endings of jaw muscle spindle thus appear to accurately "encode" jaw position when movement velocity is less than about 20mm/sec.

The static position-sensitivity of primary afferents was extremely variable, both within and between cells. The relationship between firing rate and jaw movement velocity was very non-linear: for jaw-opening movements at rates exceeding a few mm/sec, primary endings fired at what appeared to be their maximal rate, whereas for all closing movements at rates exceeding a few mm/sec, the afferents fell silent or fired at a low, irregular rate. While the firing rate of primary endings was not related, in a regular or linear manner, to movement velocity, large changes in the firing rates of these afferents precisely "signaled" the time at which the jaw began to move up or down.

These results are interpreted to be supportive of a growing body of evidence that muscle spindle afferents are important sensory receptors that provide the CNS with information about the position and movement of limbs.

124.8 AN ELECTRON MICROSCOPIC ANALYSIS OF THE LEFT PHRENIC NERVE IN THE RAT.
L.A. Langford and R.F. Schmidt. Physiologisches Institut der Universität, D-2300 Kiel, Fed. Rep. of Germany.

The rat phrenic nerve-diaphragm preparation is used extensively in physiological studies and the present trend of thought is the ratio of motor to sensory axons is 2:1; yet, these figures are based on light microscopic studies which cannot adequately resolve all unmyelinated axons (Hinsey, Hare and Phillips, Proc. Soc. exp. Biol. 41: 411-414, 1939). The purpose of this study is to quantitatively categorize all axons in the phrenic nerve by using ablative surgeries and counting the remaining axons in an electron microscope. Left phrenic nerves from normal and operated rats were prepared for EM analysis according to the method of Langford and Coggeshall (Anat. Rec. 197: 297-303, 1980). Since the motor and sensory outflow to the phrenic nerve originates from segments C_3-C_6, left dorsal root ganglionectomy (C_2-C_8), left intradural ventral rhizotomy (C_2-C_8) or cervical sympathectomy were done. All axons, myelinated and unmyelinated, were counted from gold sections prepared from a 4 mm segment of left phrenic nerve which had been removed 3 mm rostral to the diaphragm.

The averages of the preliminary counts are as follows:

		MYEL	UN	TOTAL
NORMAL	n=10	404	300	704
DRG	n= 4	299	117	416
VR	n= 4	135	247	382
SYMP	n= 4	386	226	612

In conclusion, 57% of the phrenic axons are myelinated and 43% are unmyelinated. In regard to the myelinated axons, 299 (69%) come from the ventral roots and 135 (31%) from the dorsal root ganglia. Of the unmyelinated axons, 183 (59%) stem from dorsal root ganglia, 74 (24%) from the cervical sympathetic chain and 53 (17%) course through the ventral roots. The latter are presumably preganglionic efferents since no traces of degenerating unmyelinated axons were seen in the proximal stumps of the cut ventral roots.

Supported by the Deutsche Forschungsgemeinschaft.

125.1 PRENATAL DEVELOPMENT OF THE HUMAN SUPERIOR COLLICULUS. T. W. Robertson* (SPON: N.W. Daw), School of Optometry, Univ. of Missouri - St. Louis, Mo., 63121

The superior colliculus (SC) of the prenatal human brain was studied in autopsy material stained by the Nissl and Golgi methods. Nissl stains of 15 wk fetuses revealed a primitive lamination pattern consisting of a (1) uniformly dense superficial cellular zone and superficial light zone, (2) intermediate gradient or transitional zone, and (3) a deep light zone. Within the transitional and deep light zones, clumps or islands of high cell density were found. In the 17 wk fetus, the (1) dense superficial zone was subdivided into a subpial cellular marginal zone, a narrow light zone and a dense superficial cell zone. Large islands of high cell density were found within the anterior aspect of the dense superficial cell zone. A narrow light zone was located just beneath the dense cell zone (1), then followed by a wide transition zone (2) which contains islands of high cell density. A deep light zone (3) still remained below the transition zone. This lamination pattern was well defined within the anterior SC and poorly defined within the posterior SC. At 18 wks of prenatal age, few changes were seen in the lamination pattern of the superficial zones (1), although the narrow light zone located beneath was widened and probably represents the stratum opticum. The transition (2) and deep light zones (3) were less well defined at this age, showing rather uniform cell density. Occasional islands of densely packed cells were found in the superficial (1) and deep cell zones (3). By 22 and 25 wks, the lamination of the SC was well defined and could be divided into the seven classical laminae. This stage of development marked a period of rapid cell growth and differentiation. Many cells within the intermediate and deep gray layers at this age showed initial dendritic development.

Golgi impregnations yielded limited results in the 20 wk fetus. Deep layer "stellate like" and deep layer horizontal cells were imcompletely stained. Cell bodies of these neurons appeared lobulated. Stained dendrites were short and thick in caliber and encrusted with spines. Terminal enlargements, resembling growth cones and some filopodia were seen. By 26 wks of prenatal age, many intermediate and deep layer cells were stained. Large wide field vertical and stellate cells showed extensive dendritic growth, with many spines and filopodia. Narrow field vertical cells seemed less well developed as did piriform and horizontal cells. Tissue stained from the newborn revealed that the SC was fully differentiated by cell type, although dendrites remained encrusted with spines. Terminal swellings on some dendrites of cells in the superficial layers were found.

125.2 EVIDENCE FOR POSTSYNAPTIC REGULATION OF SYNAPTIC DENSITY IN 3-EYED FROGS. Jeanette J. Norden and Martha Constantine-Paton. Dept. of Anatomy, Vanderbilt University School of Medicine, Nashville, TN. 37232, and Dept. of Biology, Princeton University, Princeton, NJ. 08540.

One of the most intriguing questions in neurobiology is whether the number of synapses formed in a structure is regulated by presynaptic input or by the postsynaptic target site. If the number of synapses is determined by presynaptic fibers, altering the amount of presynaptic input by increasing or decreasing innervation should alter the number of synapses formed in the target structure accordingly. If the number of synapses is determined by some property of the postsynaptic site, the number of synapses formed should be relatively constant regardless of the amount of presynaptic input. To test between these hypotheses, we have applied EM methods developed for quantitating synaptic densities (Norden and Freeman, 3rd Europ. Sym. Stereol. 1: 519, 1981) to the optic tecta of 3-eyed frogs (Rana pipiens) where two of the eyes innervate a single tectum in alternating bands (Constantine-Paton and Law, Science 202: 639, 1978). To date, we have determined the numerical density (number per unit volume) of synapses in the singly and doubly innervated tecta in a total area of 75,000 um^2 in two post-metamorphic 3-eyed animals and in a normal frog of the same age. Our results indicate that the total number of synapses in singly and doubly innervated tecta of 3-eyed frogs is approximately equal, supportinng the conclusion that the number of retinal synapses formed in the optic tectum is regulated by some property of postsynaptic tectal neurons. We are currently examining the superficial tectal layers in pre-metamorphic and adult 3-eyed frogs in an attempt to quantitate dynamic changes in synaptic numbers which occur in the development and maintenance of the retinotectal projection in these animals. Supported by NIH Grants EY03718 (JJN) and EY01872 (MCP).

125.3 EYE DOMINANCE COLUMNS FORMED BY AN ISOGENIC DOUBLE NASAL FROG EYE. S.E. Fraser, C.F. Ide* and R.L. Meyer. Departments of Physiology & Biophysics and Developmental & Cell Biology, University of California, Irvine, CA 92717.

The lower vertebrate visual system can be induced to form ocular dominance columns if two eyes are allowed to co-innervate a single optic tectum (Constantine-Paton & Law, Science 202: 639). While the mechanisms responsible for the formation of these retinotectal stripes remain unknown, two prime candidates have been differences in the cell surface (right-left or animal-animal differences) and differences in the firing activity of the optic nerve fibers of the two eyes. We have tested whether cell surface differences might play a role in column formation.

Duplicate eyes were produced by removing the temporal 2/3 of a stage 31 Xenopus eyebud, leaving the nasal 1/2 fragment in situ. This fragment heals to eventually form a normal appearing eye. When the projection from this eye to the optic tectum was assayed using conventional extracellular electrophysiology, it was found to be duplicated ("double-nasal" polarity). That is, each point in the tectum received input from two positions in the visual field of the frog, arranged in mirror image fashion about the dorsal-ventral axis of the eye.

Autoradiographic techniques were used to assay the distribution of optic nerve terminals on the surface of the tectum. Following injection of the eye with 10-20 µC of ^{3}H-proline, sections of the tectum were dipped in NTB-2 emulsion and exposed at 4°C for two weeks. By electrolytically lesioning one-half of the retina before the injection of the radiolabel, it was possible to assay the optic fiber distribution of only the surviving half of the retina. In normal animals, such a procedure produces label in only those parts of the tectum expected from the known ordering of the retinotectal projection. However, this procedure demonstrated that the optic nerve fibers from each half of a duplicated eye covered nearly the entire extent of the tectum. Furthermore, the optic nerve fibers from each half of the eye had segregated into dominance columns nearly identical to those formed by three-eyed Xenopus (siblings of the nasal 1/3 animals).

The presence of dominance columns from an isogenic duplicated retina argues against any role of right-left or animal-animal differences in the formation of these columns. This is because in our experimental paradigm both halves of the retina making up the duplicated retinotectal projection are from the same side of the same animal. Thus it becomes more likely that some local phenomenon such as synchrony of nerve activity or biochemical signals that identify near neighboring fibers in the retina plays a major role in these dominance columns.

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125.4 THE COURSE OF RETINAL FIBERS IN ADULT GOLDFISH OPTIC NERVE: A WHOLEMOUNT STUDY. S.M. Fraley and S.C. Sharma. Dept. of Ophthalmology, New York Medical College, Valhalla, N.Y. 10595.

HRP was applied to selected fascicles and the axon projections through the entire optic nerve were visualized in wholemount preparations. The optic fiber projections from either the dorsal, ventral, nasal or temporal retina were studied by cutting the quadrantic axons close to the optic head, thus severing fibers peripheral to the cut. Fish were perfused with phosphate buffer 3-5 days after HRP application and the optic nerve and tectum dissected out. The nerve was reacted first with benzidine dihydrochloride then fixed and dehydrated.

Fibers from three of the retinal quadrants were relatively discreetly organized on the optic nerve. Ventral fibers formed multiple fascicles aligned parallel to each other and this arrangement was maintained throughout the length of the nerve. The fascicles were confined on the medial side of the nerve and were thus oriented towards their proper termination area in the dorsomedial tectum. Dorsal retinal fibers coursed through the inner core of the nerve. A transverse view of the nerve at the level of the optic head showed the fibers to extend from the dorsal to ventral surface as a thin band located in the center of the nerve. As the fibers projected towards the brain, a lateral displacement occurred such that at the level of the chiasma the fibers were oriented towards the ventrolateral side of the tectum. Temporal retinal fibers projected in several parallel running fascicles located primarily on the lateral side of the nerve. The tectal innervation of temporal fibers appears to be complex and preliminary data suggest that some component of these fibers bypass the optic tracts to enter the rostral tectum directly. Nasal retinal fibers were less discreetly organized on the optic nerve in that the projection overlapped those of the other quadrants. Nasal fibers travelled in two major bundles which were located dorsally on the nerve but which had a large amount of spread over the medial and lateral sides. These bundles cross near the level of the chiasma and innervate the tectum bilaterally. There was also greater inter-subject variability in the nasal fiber projection relative to that of other quadrants. In some fish one of the bundles showed rope-like twisting at the mid-portion of the nerve. However, in other cases, intermingling of fibers between the two bundles appeared.

The data indicate that fibers from the retinal quadrants of the eye have a gross retinotopic organization in the optic nerve. A comparison between lesions made in the central and peripheral areas of the retina were made to determine the chronotopic nerve organization.

Supported by N.E.I. 01426

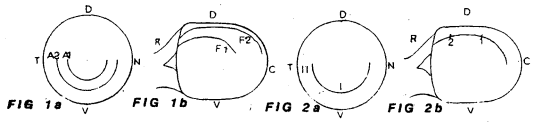
125.5 GROWTH-RELATED ORDER OF OPTIC AXONS IN GOLDFISH TECTUM. C. A. O. Stuermer* and S. S. Easter, Jr. (SPON: P. R. Johns). Div. Biological Sciences, U. Michigan, Ann Arbor, MI 48109.

Fascicles of optic axons in goldfish tectum are visible under a dissecting microscope as F1 and F2 in Fig. 1b. The fascicles to dorsal hemitectum enter rostrally, course caudolaterally, lose axons along the way, and end near the boundary between dorsal and ventral hemitecta. The experiments here were intended to reveal: 1) the retinal positions of ganglion cells contributing to individual fascicles and 2) the relation between the retinal address of an axon and the site of its exit from the fascicle.

HRP was applied to the cut ends of fascicles at various sites in dorsal tectum. Two days later, the whole-mounted contralateral retina was reacted to show retrogradely labeled ganglion cells, those with axons which passed through the HRP application site.

In all cases, the labeled ganglion cells were restricted to ventral retina in a partial annulus centered on the optic disc. This confirms and extends Rusoff (Invest. Ophth. Vis. Sci. 18 Suppl.) When fascicles were labeled rostrally, the partial annulus was roughly 180 deg in extent. Fascicles close to the boundary of dorsal and ventral hemitecta (such as F1 in Fig. 1b) gave labeled retinal half-annuli close to the optic disc (such as A1 in Fig. 1a); more peripheral fascicles (F2) gave more peripheral half annuli (A2). Thus, the fascicular order in the optic nerve and tract is maintained in the tectum; that is, axons from ganglion cells of the same age and a common retinal annulus run together. Moreover, axons from more peripheral ganglion cells occupy more peripheral regions in tectum.

When the corresponding fascicle was labeled in different animals at different sites along its tectal trajectory (as at 1 or 2 in Fig. 2b), the resultant partial annuli had different sizes, but all extended to the nasal boundary of dorsal and ventral hemiretinas. Labeling at tectal sites 1 and 2 yielded partial annuli which extended to points I and II, respectively, in Fig. 2a. It is known that the retina maps topographically onto tectum, and nasal retina projects its terminals to caudal tectum. These results indicate that all the axons destined to terminate in a particular tectal half annular zone enter the tectum in a common fascicle and exit the fascicle in order, those for rostral tectum first, those for caudal tectum later. (Supported by EY-00168 to SSE and DFG Stu 112-2 to CAOS.)



125.6 OPTIC FIBER ARRANGEMENTS IN THE VISUAL PATHWAYS OF LONG-EVANS HOODED RATS. Stuart M. Bunt and Ray D. Lund. Department of Anatomy, Medical University of South Carolina, Charleston, South Carolina 29425.

We have investigated the arrangement of fibers in the visual pathways of normal adult rats in order to compare this with animals which have received localized damage to the visual system during development.

The normal patterns were defined in part by making retinal lesions and studying the distribution of the resulting degeneration in 2 μm toluidine blue stained Epon sections. The extent of the retinal lesions were confirmed by staining the superior colliculi with the Fink-Heimer technique to reveal the pattern of terminal degeneration. In other animals HRP was applied to the retina, optic nerve or superior colliculus and the course of the HRP filled fibers traced in 40 μm sections stained with DAB or TMB. The origins of the HRP filled fibers were confirmed by flat mounting the retinae and observing the distribution of back filled retinal ganglion cells.

The degeneration studies and retinal backfills show that, immediately behind the eye, there is an orderly projection of the retina onto the optic nerve which is not quadrantic. Ventral lesions in particular give rise to a C-shaped pattern of degeneration around the edge of the nerve while other lesions tend to produce wedges or patches of degeneration running from the periphery to more central parts of the nerve. In the adult rats used in this study this pattern becomes considerably less precise as the chiasm is approached.

In the optic tract behind the chiasm the degeneration studies and tectal injections show that there is again an orderly pattern of optic fiber distribution. Fibers from more peripheral retina lie on the surface of the tract while central retina is represented more deeply. Crossing from lateral to medial across the tract, fibers are encountered originating respectively from nasal, ventral, temporal, dorsal and sometimes nasal retina again. The uncrossed retinal fibers lie in approximately the same area of the optic tract section as crossed fibers from temporo-ventral retina.

We will discuss how these fiber arrangements may affect the outcome of neonatal retinal lesions which produce anomalous ipsilateral retinal projections (Lund and Lund, Exp. Neurol., 40:377-390, '73). (Supported by NIH Grant EY03414).

125.7 FIBER ORDER IN THE RETINOTECTAL PATHWAY OF RANA PIPIENS. T.A.Reh, E.C. Pitts, and M. Constantine-Paton, Biology Department, Princeton University, Princeton NJ 08544

In order to determine the arrangement of the fibers in the optic nerve and tract of the frog, Rana pipiens, injections of horseradish peroxidase (HRP) were made in one of three positions along the visual pathway: in the retina, in the tectum or in the optic chiasm. After survival periods of from one to four days the brains were processed as whole mounts allowing us to follow small bundles of fibers throughout their course from the retina to the tectum.

Injections of HRP into the optic chiasm or tract produce full or half circles of labelled axon terminals in the tectum, and corresponding patterns of HRP filled retinal ganglion cells (RGCs), suggesting that fibers in the frog optic chiasm and tract are organized into age-related bundles as is found in teleosts. Unlike teleosts, however, the frog optic nerve contains an isomorphic representation of the pattern of label in the retina and tectum. That is, when an injection into the chiasm produces a circle of labelled terminals in the tectum and a circle of labelled RGCs in the retina, it also produces a circle of labelled axons in the optic nerve. Additional HRP injections into the poles of the retina and tectum filled bundles of axons in specific positions in the nerve, making it possible to determine the axial alignment of the visual map in the nerve. We found that the axes of the nerve are aligned like those in the tectum instead of in the manner found in the retina. Indeed, sections through the optic nerve head reveal a marked degree of fiber crossing such that the nasal/temporal axis is reversed with respect to the dorsal/ventral axis as the fibers leave the eye.

In sum, we have found a high degree of order in the frog visual pathway. As the RGC axons exit the eye, they rearrange to invert one axis while the orientation of the other is preserved. The axons travel through the nerve in a "tectotopic" fashion and bundle into age specific fascicles at the optic chiasm. Subsequent to the chiasm, these bundles split in two forming the medial and lateral optic tracts.

Supported by NSF Grant BNS-8110225

125.8 EVIDENCE FOR SLIDING CONNECTIONS IN THE DEVELOPMENT OF THE RETINOTECTAL PROJECTION IN THE CHICK. S.C. McLoon. Dept. of Anatomy, Medical University of South Carolina, Charleston, S.C. 29425.

Earlier studies have suggested the first retinal axons to arrive on the tectum grow over the tectal surface and penetrate in its center. However, we have found a wave of cell death in the developing ganglion cell layer which proceeds from temporal to nasal retina suggesting that synaptogenic events in the tectum progress from rostral to caudal rather than central to peripheral. In this study we have re-examined the pattern of ingrowth and penetration of the retinal projection using a newer more sensitive technique. Embryos at three days of incubation were transferred to embryo culture chambers to facilitate access. Embryos at each embryonic age from 6 through 12 days received an injection of 2 μl WGA conjugated horseradish peroxidase into one eye. After an appropriate survival period, which ranged from six to 24 hours, the brains were fixed, sectioned and processed for HRP histochemistry using tetramethyl benzidine as the chromogen. The tectum contralateral to the injected eye was reconstructed from the serial sections using computer-graphic techniques.

The first retinal fibers arrive on the tectum on embryonic day six (E6). The fibers enter at the rostral-ventral tectum. By late E6 the first evidence of penetration of label into the tectal layers below the superficial fiber layer is apparent. Penetration follows closely behind the advancing front of growing axons. During E7 and E8 the retinal projection grows dorsally to fill the most rostral extent of the tectum. From E9 through E12 the retinal axons grow caudally to cover the remaining tectum. During this entire period of growth the optic fibers penetrate the tectal layers in an area shortly after reaching that area. Electron microscopic examination of the tectum shows that early synapses, with asymmetric junctions and clusters of round synaptic vesicles, appear temporally and spatially in the same pattern as the penetration of the optic fibers.

These results show that the retinotectal projection develops in approximately a rostral-ventral to caudal-dorsal pattern. This appears to be similar to the pattern of histogenesis and cellular maturation of the tectum (LaVail and Cowan, '72). Since the first ganglion cells arise in central retina and the first connections are to rostral tectum, it could be suggested that an early projection from central retina to rostral tectum must "slide" to its ultimate site of termination in central tectum. One other possibility which we are currently pursuing is that axonal outgrowth from the retina may not follow the pattern of histogenesis in the retina. Thus if the first axons leaving the eye arise from temporal retina, sliding connections in the tectum would not be necessary.

(Supported by grant EY03713 from the NIH.)

125.9 EMBRYONIC RETINAE TRANSPLANTED TO THE INFERIOR COLLICULUS OF NEWBORN RATS. L.K. McLoon and R.D. Lund. Dept. of Anatomy, Medical University of South Carolina, Charleston, S.C. 29425.

Embryonic retinae transplanted to the superior colliculus of newborn rats project into the superior colliculus and other subcortical visual nuclei but make no connections with non-visual structures (McLoon and Lund, Exp. Brain Res., 1981). If a retinal transplant is placed over an intact or deafferented non-visual nucleus close to the superior colliculus, will the transplants make aberrant connections with a non-visual nucleus and will the transplants make connections with the superior colliculus and other visual centers even without direct contact or damage to those areas?

This was tested by transplanting neural retina removed from embryonic day 14 days over or into the caudal end of the inferior colliculus of newborn rats. Unilateral lesions of auditory cortex were made by aspiration. In half the animals unilateral eye enucleations were also performed.

One month after transplantation, the retinal transplants were located and injected with HRP. The transplants matured and showed the typical laminar pattern seen previously. Labeled axons left the transplants in bundles and were followed forward through the inferior colliculus into the superior colliculus, where they formed local terminal ramifications. These ramifications were more extensive when the eye providing major innervation to the superior colliculus had been removed. Thus far labeled axons could not be traced beyond the rostral border of the superior colliculus. Fibers were also seen running between the transplants and the inferior colliculus, but it is not clear yet whether these are anterogradely or retrogradely filled.

These results show that it is not necessary to damage a region for a transplant to make connections with it; the retinal axons are still able to find their way to an appropriate visual nucleus, the superior colliculus. It remains to be seen whether these transplants make aberrant connections in an atypical fashion with non-visual structures, in particular with ones which have been deprived of certain natural afferents at the time of transplantation.

(LKM is NEI postdoctoral fellow EY05394. Supported by EY03326.)

125.10 TRANSPLANTATION OF EMBRYONIC NEURAL TISSUE TO ADULT RAT VISUAL SYSTEM: A COMPARISON WITH STUDIES USING NEWBORN HOSTS. R.D. Lund and S.C. McLoon. Department of Anatomy, Medical University of S.C., Charleston, S.C. 29425.

Our earlier studies showed that embryonic retina, cortex or tectum when transplanted adjacent to the superior colliculus (SC) of newborn hosts differentiate into structures showing many of the normal histological features of each donor region. They all send projections into the host brain, and cortex and colliculus, at least, receive host afferents. In this study we have examined how the same regions behave when placed on or in the SC of adult rats. Outbred Long-Evans hooded rats were used. The techniques were similar to those used previously, except that an area of cortex was removed to expose the host SC. The surface of the SC was damaged to ensure maximum apposition of host and donor tissues and one eye was removed in some rats to encourage optimal opportunity for transplant innervation of the host. Projections were studied after 2-3 months survival using HRP and/or autoradiography.

In comparison with transplants to newborns, the following conclusions may be made: (1) A high percentage of transplants survive. (2) They do not appear as well differentiated histologically as after transplantation to newborns. (3) Axons from each region grow as much as 2 mm into the host brain from the point of penetration, and have terminal arbors in the superficial layers of SC. The fine details of the terminal arbor appear to differ according to the donor tissue used. (4) In marked contrast to transplants to neonates, orthograde studies show no significant projection from the host eye even to tectal transplants embedded in host SC. (5) Retrograde labeling studies show no evidence of a host projection into the transplants, again in contrast to neonate transplants.

In summary, while transplant axons do ramify in the host SC, the vigorous growth of axons connecting host and transplant using newborn hosts is not seen with adult recipient. (Supported by grant EY03326 from NIH).

125.11 PROJECTIONS OF GROWTH-CONE-BEARING FIBERS OF RETINAL GANGLION CELLS IN CULTURE: EARLY BRANCHING DEPENDS ON AGE OF TECTAL TARGET EXPLANT. D.R. Friedlander and S.M. Crain. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Half retinas and superior colliculi (tecta) from 13-14 day mouse embryos were explanted on a collagen substrate, separated by a 0.5 mm gap (Smalheiser et al., Brain Res. 204,'81). After 5 days in vitro, retinal ganglion cells were labeled by extracellular ionophoresis of HRP into the optic nerve-head region, followed by a sensitive DAB histochemical procedure prior to fixation. Cleared co-cultures were studied as whole mounts. Cell bodies with dendritic arbors stained throughout the retinal ganglion cell layer. Bundles of ganglion cell axons, generally exhibiting smooth, unbeaded morphology grew out of the retina and invaded the tectal explant. Most of the darker staining fibers, both on the substrate and in the target tissue, terminated in growth cones. Occasionally, backfilled tectal cells were observed, but their poorly-stained neurites could be clearly distinguished from retinal axons.

The projection of retinal growth-cone-bearing fibers within the target was analyzed with a 40X water-immersion objective and a camera lucida. Fibers were classified into 3 groups, according to the complexity of their branching. Simple (S) axons were unbranched. Intermediate (I) fibers exhibited one or more bifurcations. Complex (C) axons had branches of second or higher degree, or closely spaced repeated primary branches, and resembled terminal arborizations in embryos (e.g. Lazar, J. Anat. 116,'73) and in older co-cultures (Smalheiser et al., ibid).

Growth-cone-bearing fibers were studied in standard co-cultures (14 explants), or in cases where tectum had been explanted 2 weeks prior to retina (7 explants). Analysis of the pooled data showed that the heterochronous cultures had more complex and intermediate fibers (S=52%, I=28%, C=13%; N=135) than the synchronous explants (S=82%, I=15%, C=3%; N=149). Cultures in which retinas were explanted 1 week after tecta (5 explants) exhibited intermediate values (S=70%, I=20%, C=9%; N=44). Retinal axons growing into an inappropriate target, spinal cord (23 explants), branched rarely (S=97%, I=1.5%, C=1.5%; N=67).

These observations suggest that older tecta facilitate branching of ingrowing retinal fibers, although other alterations during in vitro development of tectum must be evaluated. It will be interesting to determine whether the increased branching of retinal fibers growing in older tecta is correlated with enhanced development of functional connections.

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125.12 REPRESENTATION BY EACH EYE OF THE VISUAL SPACE IN THE OPOSSUM'S SUPERIOR COLLICULUS: FUNCTIONAL ASPECTS AND IMPLICATIONS FOR STUDIES ON NEUROPLASTICITY. A.S. S. Ramôa*; C.E. Rocha-Miranda*; E. Volchan*; L. C. Gawryszewski* (SPON: L.R.G. Britto). Instituto de Biofísica da UFRJ, and Departamento de Fisiologia da UFF, Rio de Janeiro, Brazil.

Visual receptive fields and magnification factors were analysed for each eye in penetrations along the A-P axis of the optic layers of the opossum's superior colliculus. The collicular regions where the ipsilateral and contralateral binocular regions are represented (rostral pole (RP) and direct binocular region (DBR), respectively) were explored at or close to the representation of the horizontal meridian (Volchan, E. et al., Exp. Brain Res., in press). Of 242 units, half had receptive field centers of the contralateral eye at the ipsilateral hemifield (RP units) and the other half at the contralateral hemifield (DBR units). In RP units a large fraction of the receptive fields of the ipsilateral eye straddled the vertical meridian (VM) or were confined to the contralateral hemifield (24 out of 26). Only 50 out of 121 RP units responsive to the contralateral eye conformed to the first condition ($X^2 = 23.7$, $p < 0.005$). As a result of the tendency for the receptive fields of the ipsilateral, but not the contralateral eye, to overlap the VM, progressively more rostral recordings yield an increase in the binocular convergent disparity as a function of the eccentricity for the receptive field of the contralateral eye ($r = 0.64$, $p < 0.01$). Since the disparity didn't vary with eccentricity in the DBR units, we suggest that stimuli at the midline and close to the animal might be the natural ones to drive large disparity units present in the RP.

In accord with the VM's expanded representation for the ipsilateral eye at the single unit level, the values of the magnification factor for each eye, determined by multi-unit recordings, aren't congruent in all the extension of the colliculus. At the RP the magnification factor for the ipsilateral eye is greater than that for the other eye. This must be taken into account when analysing the expanded retinotectal projection on the side of the remaining eye subsequent to early enucleation. (Supported by CEPG/UFRJ, FINEP/B-76/81/150/0000 and CNPq (Proc. 40.1636/81).

125.13 RECEPTIVE FIELD PROPERTIES OF TECTAL CELLS IN IGUANA *IGUANA*. Neal S. Gaither* and Barry E. Stein. (SPON: J. Astruc). Dept. of Physiology, Med. Coll. of Va., Richmond, VA 23298.

In a previous report (Stein, B.E. and Gaither, N.S., J. Comp. Neurol., 202: 69, 1981) we compared many of the organizational features of the sensory and motor representations in the reptilian optic tectum and the mammalian superior colliculus (SC). The organizational similarities in these homologous structures were striking and prompted us to extend these comparisons. Therefore, in the present series of experiments we studied the receptive field properties of tectal cells in the iguana. This was done in the same way as we had previously studied SC cells in mammals so that direct comparisons between reptiles and mammals could be made.

A total of 148 tectal cells was studied. Once again basic similarities between tectal and SC cells were evident. Visual cells (n=105) of the tectum responded in a phasic fashion even to maintained stimuli. Most (82%) of those tested (n=59) were ON-OFF or OFF throughout their receptive fields, although a few (n=7) did have small regions of the border in which responses differed from those of the rest of the field. Many visual cells of the tectum exhibited spatial summation and/or spatial inhibition within the borders of their 'excitatory' receptive fields and had suppressive surrounds as well. In addition, velocity and directional selectivity was exhibited by these cells and interactions between these parameters of movement could usually be demonstrated. Somatic cells in the tectum also responded only in phasic fashion, were primarily cutaneous and fatigued readily to repeated stimuli. These characteristics of visual and somatic tectal cells are basically the same as those exhibited by SC cells.

Tectal visual cells differed from visual SC cells in at least two respects. Unlike SC cells, tectal cells preferred stationary to moving stimuli, and the presence of specific receptive field properties was related to receptive field eccentricity. Cells with receptive fields beyond the central 5^{o} of the visual field were best adapted for the detection of movement and coding the parameters of movement (velocity and direction). Receptive fields within the central 5^{o} of the visual field seemed poorly suited for these tasks, but well adapted for the analysis of the spatial features of stationary targets.

Despite differences in the distribution of cell types, the physiological profiles of tectal and SC cells appeared to have fundamentally similarities. It seems likely that these extensive similarities in the organization and receptive field properties of tectal and SC cells represent the retention of ancestral characteristics in very different extant species.

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125.14 ULTRASTRUCTURAL STUDIES ON THE TIME COURSE OF INVASION AND SYNAPTOGENESIS BY REGENERATING OPTIC FIBERS IN THE OPTIC TECTUM OF ADULT GOLDFISH. Jeffrey D. Radel and Myong G. Yoon. Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada B3H 4J1

The time course of retino-tectal synapse formation after optic nerve crush in adult goldfish (9cm, 20^{o}C) was studied. At the onset of the time series experiment, the left optic nerve was crushed in the first group of fish. Three days later, the right optic nerve was also crushed in the first group and the left optic nerve was crushed in the second group of fish. Sequential crushing, at 3 day intervals, of the left optic nerve followed by the right optic nerve was performed over 14 time points, up to 42 days. On day 45, all fish received bilateral intraocular injections of ^{3}H-proline, and were sacrificed 10 hours later. The tecta were dissected free and processed for both light microscopic autoradiography and electron microscopic examination of the ultrastructures at given time points.

In a normal goldfish, the optic fiber terminals are characterized by pale mitochondria, spherical clear synaptic vesicles (49-53 nm diameter) and multiple (2 to 4) synaptic contacts. Eighteen days after optic nerve crush (18 dpc), fibers containing pale mitochondria were found to initially reappear in the stratum opticum (SO) within 700 µm of the rostral pole. This coincides with the earliest sign of tectal invasion by ^{3}H-labelled regenerating optic fibers as revealed in autoradiographs. At 21 dpc, fibers containing pale mitochondria formed fasicles in the SO and dipped into the stratum fibrosum et griseum superficiale in the left tectum. At 24 dpc, newly formed retino-tectal synapses were found in the right tectum of the same fish; each regenerating optic fiber terminal, containing pale mitochondria and spherical vesicles, formed 2 or 3 synaptic contacts, and often appeared near degenerating debris. The regenerated retinal fibers showed uniform distribution of vesicles throughout the terminal area (up to 39 dpc). This contrasts with the localized distribution of vesicles along the presynaptic membrane in normal goldfish.

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125.15 AN IN VITRO EXAMINATION OF ENHANCED MITOSIS IN THE DEEP LAYERS OF THE GOLDFISH OPTIC TECTUM DURING REINNERVATION BY REGENERATING OPTIC FIBERS. Deborah B. Henken and Myong G. Yoon. Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada B3H 4J1

Previous studies in vivo show that a class of radial glial cells, whose nuclei are located between the periventricular and ependymal layers, undergo mitosis in the optic tectum of adult goldfish. The rate of their mitoses is specifically enhanced during a period between 4 and 5 weeks after crush of the contralateral optic nerve. At about this time, the optic tectum is reinnervated by regenerating optic fibers. This temporal coincidence suggests that regenerating optic fibers may exert mitogenic effects on these radial glia. To study the mitogenic interaction, we have devised an in vitro technique, which will allow biochemical manipulations not attainable in vivo.

Thirty five days after unilateral optic nerve crush goldfish were deeply anaesthetized. The entire optic tectum was dissected free, bisected perpendicular to the rostrocaudal axis, and submerged in a modified Leibovitz L-15 culture medium. To label mitotic cells, tritiated thymidine (^{3}H-Tdr) was introduced into the medium for predetermined durations ranging from 30 to 120 minutes.

The ratio of labelled cells in the experimental tecta (contralateral to the crushed nerve) to that of the control side (ipsilateral) increases linearly from approximately 1 to a maximum of 5 as the ^{3}H-Tdr pulse duration increases from 30 to 75 minutes. At 90 minutes, the ratio declines rapidly and returns to 1. This observation in vitro agrees with that found in vivo. Hence, the in vitro technique of labelling mitotic cells can be used for a biochemical investigation of the factors involved in the induction of radial glial mitosis by regenerating optic fibers.

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125.16 INTEROCULAR TRANSFER AND VISUAL ANGLE ANALYSIS IN GOLDFISH WITH UNILATERAL RETINOTECTAL COMPRESSION. Ambrose A. Dunn-Meynell* and George E. Savage* (SPON: A.Rothballer). Dept. of Zoology and Comparative Physiology, Queen Mary Coll., Univ. of London, London E1 4NS, England.

Retinotectal compression in the horizontal axis of the visual field of one eye was induced by unilateral caudal tectal ablation in 3 - 4½ inch goldfish, followed by 5 to 11 months recovery. These subjects were classically conditioned to discriminate between 3 and ¾ cm horizontal bars, training monocularly via the eye contralateral to the operated tectum. Conditioned cardiac deceleration was used as a measure of learning. During testing for interocular transfer, 6, 3, 1½, ¾ and ⅜ cm horizontal bars were presented to the eye contralateral to the intact tectum. Other subjects with retinotectal compression were trained with 3 and ¾ cm vertical bars, and tested for interocular transfer with the above range of 5 bar lengths presented vertically instead of horizontally.

Operated subjects learnt the vertical bar length discrimination at a similar rate to unoperated controls trained with the same task, and showed similar responses to the controls to stimuli used in interocular transfer testing. Operated subjects trained with the horizontal bar length discrimination learnt more slowly than, and gave different results on interocular transfer to, the controls trained with the same horizontal length discrimination. However the learning rate and responses on interocular transfer were similar between operated subjects trained with the above horizontal bar length discrimination, and controls trained with a 1½/⅜ cm horizontal bar length task and tested for interocular transfer with the normal range of stimuli.

The following conclusions may be drawn about subjects with retinotectal compression in the horizontal axis of one visual field:

a) Learning occurs (as other workers have shown).
b) Interocular transfer occurs from the operated to the intact brain half in an apparently normal fashion.
c) Visual angle analysis in the horizontal axis of the visual field appears to be abnormal, though vertical visual angle analysis seems normal.
d) The results for operated subjects trained with horizontal bars are more similar to results for controls trained with a 1½/⅜ cm than with a 3/¾ cm discrimination. Therefore it appaers that in this task, visual angles are analysed according to tectal length of visual projection, without compensation being made in the analysing mechanisms for the changes in retinotectal magnification factor accompanying retinotectal compression.

(Supported by Science Research Council grants).

126.1 CORRELATION BETWEEN ANTICONVULSANT ACTIVITY AND BENZODIAZEPINE RECEPTOR BINDING. Andrew Y. Chweh*, Ewart A. Swinyard*, and Harold H. Wolf* (SPON: Stuart A. Turkanis). Department of Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Four selected benzodiazepines (BDZ), each with a distinct profile of anticonvulsant activity, were tested for their affinity for the BDZ receptor and/or ability to inhibit adenosine uptake. Anticonvulsant potencies in mice (i.p.) were determined at the time of peak effect by the maximal electroshock (corneal electrodes, 50 mA, 60 Hz, 0.2 sec.) and sc strychnine (1.20 mg/kg) seizure pattern tests and sc pentylenetetrazol (PTZ, 85 mg/kg), sc bicuculline (2.70 mg/kg), and sc picrotoxin (3.15 mg/kg) seizure threshold tests. The anticonvulsant potencies of the BDZ, as measured by the PTZ test, correlate well with their respective BDZ receptor binding potencies; however, there is no correlation between anticonvulsant potency, as measured by the other four tests, and BDZ receptor binding potencies. The inhibitory potencies of the BDZ, as measured by adenosine uptake, did not correlate well with any of their anticonvulsant potencies. In addition, pentylenetetrazol (0.2-1.0 mM) did not alter adenosine uptake by purified mouse whole brain synaptosomes. These results further support the concept that benzodiazepine receptor binding correlates well with the anti-PTZ activity of benzodiazepines. (Supported by NIH Contract No. NO1-NS-1-2347.)

126.2 BENZODIAZEPINE RECEPTOR DECLINES IN HIPPOCAMPUS FOLLOWING LIMBIC SEIZURES. V.M.B. Kraus*, R.M. Dasheiff, R.J. Fanelli, J.O. McNamara (SPON: J.L. Giacchino). Depts. of Med. and Pharm., Duke Univ.; and Epilepsy Ctr., VA Med. Ctr.; Durham, NC 27705.

Benzodiazepines (BZs) are known to be effective anticonvulsants of limbic seizures in animals and man. We therefore hypothesized that BZ receptors (BZRs) may be altered in an animal model of limbic seizures. To test this hypothesis, we used a model of limbic seizures induced by electrolytic lesions of the entorhinal cortex (EC) (Brain Res. 231:444-450, 1982). This projection is the primary afferent to hippocampal formation (HPF), the immediate and principal target being dentate granule cells (DGC) located in the dentate gyrus. An electrolytic lesion of the EC was done unilaterally in adult male Sprague-Dawley rats, controls were sham operated, all were sacrificed 72 hours later. BZRs were measured in vitro with [^{3}H]-Flunitrazepam binding (mean of fmol/mg protein $\pm$ SEM) in membranes prepared from microdissected HPF (dentate gyrus, regio inferior, regio superior). Data ipsi- and contralateral to lesion were not significantly different; therefore results were pooled and analyzed with a two-tailed student's t-test. Significant bilateral and symmetrical declines in BZR binding occurred only in dentate gyrus: Control (n = 22) 762 $\pm$ 40, Lesion (n = 22) 590 $\pm$ 45, (23%, $p < .01$). Scatchard plots of binding isotherms show reductions in receptor number without change in affinity.

Lesion and control animals were treated with phenobarbital (40-80 mg/kg/day) daily for two days prior and two days following surgery then sacrificed on the third day. These doses blocked the appearance of limbic seizures but not the degeneration of axons due to EC lesioning (Brain Res. 235:327-334, 1982). Phenobarbital treatment blocked the BZR declines: Control 658 $\pm$ 81, Lesion 648 $\pm$ 83.

The results confirm our hypothesis and indicate that BZRs are decreased in association with these limbic seizures. The receptor decline cannot be attributed simply to loss of EC axonal terminals, since the decline was bilateral and the EC projection is almost exclusively ipsilateral. The receptor decline found here stands in direct contrast with receptor increases we have reported with two other models of seizures, amygdala kindling and repeated electroshock (P.N.A.S. 77:3029-3032, 1980). The reason for this difference is unclear.

The reduced numbers of BZRs almost certainly reside on DGC, since previous work from this laboratory has localized BZRs in dentate gyrus exclusively to DGC (P.N.A.S. 79:193-197, 1982). These receptor declines may translate into decreased endogenous recurrent inhibition (a presumed GABA-ergic synapse) of DGC which could lead to their excessive excitability. Thus these BZR declines may cause or facilitate limbic seizures.

126.3 ^{3}H DIAZEPAM BINDING WITH THE DEVELOPMENT OF AMYGDALOID KINDLING E. I. Tietz and R. F. Berman. Department of Psychology and the Neuroscience Program, Wayne State University, Detroit, MI 48202.

Increases in benzodiazepine (BZ) receptor number (Bmax) have been demonstrated following spontaneous and experimentally induced seizures (Syapin et al., 1981; Paul & Skolnick, 1978), including the kindling model (McNamara et al., 1981). The progressive changes in the efficacy of diazepam as an anticonvulsant with the development of kindling (Albertson et al., 1981) suggests a gradual alteration in the function of the BZ receptor corresponding to the development and generalization of a seizure focus. The present study analyses changes in BZ binding over the course of kindling.

Male Long-Evans rats were stereotaxically implanted with bipolar electrodes in the amygdaloid region. Afterdischarge (AD) thresholds were obtained by incrementing electrical stimulation until a 2 sec AD was elicited ($\bar{X}$=86.9$\pm$.45 S.E. µamps). Rats were sacrificed for binding assay immediately or 24 hr after the initial AD, or immediately or 24 hr after a Stage 3 or Stage 5 kindled seizure. Non-implanted control animals were sacrificed with each experimental animal. Immediately after sacrifice the brains were removed and the area of the amygdala-entorhinal cortex and the hippocampus were each dissected, homogenized, and fractionated by centrifugation. A P_2 pellet was obtained and washed 3 times. Protein concentrations were adjusted to .25 mg/ml, and samples were frozen until use. ^{3}H Diazepam binding was carried out in triplicate in the presence or absence of 1 µM diazepam. Scatchard analyses were used to compare experimental animals with their matched controls.

No changes in the number of BZ receptors were found immediately or 24 hr after the initial AD in either neural region examined. However, increases in receptor number were seen in the amygdaloid region (23%), but not in the hippocampus immediately after a Stage 3 seizure. No changes in receptor number were found 24 hr after a Stage 3 seizure for either region. A larger increase in Bmax was found following a fully kindled stage 5 seizure. This increase was noted in both the amygdaloid region (17%) and the hippocampus (34%) and remained evident in the hippocampus (34%), as previously reported (McNamara et al., 1981) and the amygdala (32%) 24 hr later. No changes in K_D between kindled and control animals were found for any time point or neural region examined.

These results indicate that changes in BZ receptor number can be demonstrated with intermediate levels (Stage 3) of amygdaloid kindling. However, these initial changes are transient in nature and do not persist until generalized (Stage 5) seizures have developed.

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126.4 REGULATION OF BENZODIAZEPINE RECEPTORS IN FASCIA DENTATA BY CHLORIDE, BARBITURATE, AND GABA: EFFECTS OF REPEATED KINDLED SEIZURES. R.J. Fanelli and J.O. McNamara, Depts. of Med. and Pharm., Duke Univ.; and Epilepsy Ctr., VAMC, Durham, NC 27705.

We previously found that kindled seizures result in increased numbers of benzodiazepine receptors (BZRs) in fascia dentata membranes localized to granule cells. To provide a framework for correlative electrophysiologic investigation of this molecular alteration, we characterized the regulation of BZR binding by NaCl, pentobarbital (PB) and GABA. Kindled seizures (K) were induced by daily administration of low levels of electrical current to the right amygdala. Control (C) animals underwent electrode implantation but received no stimulation. K treated animals were sacrificed 24 hours after the last seizure with a matched C. Binding was measured in well washed freeze-thawed membranes prepared from fascia dentata.

In all experiments, the [^{3}H] flunitrazepam (FLU) binding in membranes from K treated animals exceeded that in C by 24%. NaCl (100 mM) increased BZ binding in membranes prepared from C and K treated animals by roughly equivalent amounts (19% and 14% respectively). The effects of PB or GABA were subsequently determined in the presence of NaCl (100 mM); henceforth binding with NaCl will be referred to as basal binding. Nine concentrations of PB (5×10^{-6} - 10^{-3} M) were examined in seven experiments. In each experiment the percentage of basal binding in membranes from C and K rats was plotted as a function of PB concentration. If the BZRs in membranes from K treated rats were coupled to barbiturate receptors in a pattern identical to C rats, then the PB effect in the K and C rats, when expressed as percentage of basal binding, should be equivalent. The averaged data from all seven experiments disclosed that the curve of the K treated animals slightly exceeded the C curve but the difference was not statistically significant. Six concentrations of GABA (10^{-7} - 10^{-4} M) were employed in five experiments. The data were analyzed identically as described in the PB experiments above. The GABA induced increase of the K treated animals was considerably less than predicted (paired t, 2-tailed, $p < .001$).

These data provide evidence for a differential regulation of BZ binding by GABA and PB in membranes from K treated animals. The PB effect did not significantly differ from the predicted value whereas the GABA effect was significantly less than predicted. The attenuation of the GABA response cannot be due to increased amounts of GABA in the binding media of K, since measurement of GABA excluded this possibility. The reduced response to GABA may reflect a desensitized state of this GABA receptor in granule cell membranes of K treated rats. Alternatively the increased BZR binding in the K treated animals may reflect the appearance of "new" BZRs which cannot be regulated by added GABA.

126.5 LONG-TERM DOWN REGULATION OF BENZODIAZEPINE RECEPTORS FOLLOWING AMYGDALA KINDLING. H.B. Niznik*, S.J. Kish* and W.M. Burnham (SPON: O. Hornykiewicz). Dept. of Pharmacology, University of Toronto and the Human Brain Lab, Clarke Institute of Psychiatry, Toronto, Canada.

Adult, male Royal Victoria hooded rats (220-250 g) were implanted with bipolar electrodes in the right amygdala, and kindled daily (1 sec, 60 Hz, biphasic, 1 msec pulses at 400 μA peak-to-peak) until 6 consecutive stage 5 seizures were evoked. Implanted but unstimulated controls were handled in a similar manner. Fifteen or 60 days following the last stimulation, rats were decapitated, their brains rapidly removed and frozen on dry ice. The hypothalamus, right and left regions of the cortex, striatum, amygdala, and dorsal hippocampus were subsequently dissected. Saturation isotherms of ^{3}H-flunitrazepam (^{3}H-FLU) binding were generated for each region according to the method described by Placeta and Karobath (1979). B_{max} (number of receptors) and K_d (affinity) values were estimated from Scatchard plots of the saturation binding data by linear regression analysis. All values are the mean (± SEM) obtained from 6-7 animals. Data were analyzed by Student's t-test.

A 20% reduction in the B_{max} (fmoles/mg tissue) of ^{3}H-FLU binding was observed in the hypothalamus (control 80±3, kindled 64±2) and right cortex (control 150±6, kindled 120±7) of kindled rats following a 15-day seizure-free period ($p<.01$). In addition, small bilateral, but non-significant reductions (8%) in ^{3}H-FLU binding were noted for both the hippocampus and amygdala. No significant alterations of the $K_{d(app)}$ were seen in any of the regions studied. These receptor changes are relatively permanent since ^{3}H-FLU binding was still decreased by 20% in the hypothalamus (control 95± 4, kindled 76± 3) and right cortex (control 162± 7, kindled 131± 5) 2 months following the last convulsion ($p<.01$).

Previous studies have reported short-term (24 hr) elevations of benzodiazepine (BZD) receptors following amygdaloid-kindled seizures. These receptor alterations may be related to post-ictal inhibitory mechanisms since similar receptor increases are observed immediately following either ECS or metrazol induced convulsions. The present data indicate that in addition to these changes, kindled seizures induce long-term reductions in BZD binding which can be seen once compensatory post-ictal mechanisms are attenuated.

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126.6 STIMULATION OF ^{3}H-DIPHENYLHYDANTOIN BINDING BY DIAZEPAM : POSSIBLE INVOLVEMENT OF PHOSPHOLIPID METHYLATION, J. P. Chambon*[1], D. S. Shah*[2] and A. Guidotti[2]. 1)Centre de Recherches Clin-Midy, Groupe Sanofi, 34082 Montpellier Cedex, FRANCE. 2)Lab. Preclinical Pharmacol., Nat. Mental Health, St-Elisabeth Hosp., Washington DC 20032.

Specific binding sites for ^{3}H-diphenylhydantoin (^{3}HDPH) in rat brain membranes have been described (Shah et al., Neuropharm. 20, n°11, 115-119, 1981). These studies revealed a marked dose-dependant increase (up to + 200 p.cent) of ^{3}HDPH specific binding when experiments were carried out in the presence of benzodiazepines at concentrations ranging from 0.1 to 100 μM. This effect occurred in brain but not in peripheral tissues such as liver or heart and is in good agreement with in-vivo pharmacological studies demonstrating a potentiation by diazepam of DPH antagonism of electroshock-induced seizures in mice. Addition of substrate of transmethylase I and II, S-adenosyl methionine (SAM) 2 10^{-4} M to the incubation medium in routine assays (30 min of incubation at 37°C followed by 30 min of incubation at 0°C using a final concentration of ^{3}HDPH of 2.4 nM) resulted in a moderate increase (up to 50 p.cent) of ^{3}HDPH specific binding ; in contrast when SAM 2 10^{-4} M was added in the same experiment together with diazepam 5 μM, a strong increase of stimulation of DPH binding by diazepam was revealed (up to 170 p.cent). Scatchard analysis of the saturation isotherms for ^{3}HDPH binding revealed a large increase in Bmax (950 fmol/mg prot. in controls ; 2175 fmol/mg prot. in experiments with diazepam 5 μM ; 5750 fmol/mg prot. in experiments with diazepam 5 μM and SAM 2 10^{-4} M) and a moderate increase of Kd. On the other hand, inhibitors of the methylation pathway such as 3-deaza-adenosine (3DZA) in concentrations ranging from 10^{-6} M to 5 10^{-4} M incubated with homocysteine thiolactone 1 mM induced a dose-dependant inhibition of stimulation by diazepam of ^{3}HDPH specific binding (up to 75 p.cent). No effect was observed if ^{3}HDPH binding was performed in presence of SAM + isoproterenol. In addition SAM-treated membranes did not significantly change the number and affinity of benzodiazepine binding sites. These results are consistant with the hypothesis that the potentiation of DPH action by benzodiazepines can be mediated by a stimulation of phospholipid methylation.

126.7 AMYGDALA KINDLED SEIZURES REDUCE A SELECT SUBPOPULATION OF ^{3}H-QNB BINDING SITES IN RAT DENTATE GYRUS. Daniel D. Savage and James O. McNamara. Depts. of Med. and Pharm., Duke Univ.; and Epilepsy Ctr., VA Med. Ctr.; Durham, NC 27705.

Muscarinic cholinergic antagonists bind to a homogeneous population of muscarinic binding sites. By contrast, displacement of antagonist binding by muscarinic agonists is complex and deviates significantly from simple mass action behavior. One explanation for the complexity of agonist binding is the existence of multiple affinity agonist binding sites.

Amygdala kindling is an animal model of limbic epilepsy induced by periodic electrical stimulation of the brain. We have reported a reduction in the number of ^{3}H-QNB sites in rat hippocampal formation in response to kindled seizures. We chose to examine the kindled seizure induced alteration in ^{3}H-QNB binding further by analyzing carbachol displacement (CD) curves of ^{3}H-QNB binding with a computerized curve fitting method.

Male Sprague-Dawley rats received kindling stimulations in right basolateral nucleus of amygala six times a day at intervals of 90 minutes. Twenty four hours after an animal exhibited its sixth Class 5 seizure, the stimulated animal and its unstimulated control were killed by decapitation. Hippocampal formations were removed and microdissected into hippocampal and dentate gyri. Samples were prepared for ^{3}H-QNB membrane binding assay as described before (PNAS 79:193, 1982). The binding assay consisted of 50 μg. of membrane protein and 0.3 nM l-^{3}H-QNB in a total volume of 2.0 mls of incubation buffer. Additional tubes contained either atropine (10^{-6}M) or one of fourteen different concentrations of carbachol ranging from $3x10^{-8}$M to 10^{-1}M. Specific ^{3}H-QNB binding was plotted as a function of carbachol concentration and binding data analyzed with a computerized non-linear least-squares curve fitting technique using a generalized model for complex ligand-receptor systems. The estimate of the kinetic parameters from individual CD curves were averaged and compared statistically using student's t-test.

Analysis of six CD curves from normal dentate gyrus membranes indicated that the data best fitted a two site model having a high affinity carbachol displaceable ^{3}H-QNB binding site (R_H) ($Ki_H = 6x10^{-6}$M) and a low affinity site (R_L) ($Ki_L = 4x10^{-4}$M). R_H accounted for 17% and 30% of total specific ^{3}H-QNB binding in dentate and hippocampus respectively.

Analysis of CD curves of dentate gyrus membranes from kindled seizure animals indicated a selective 26% reduction in R_L compared to control. R_H was unchanged. By contrast, kindled seizures resulted in significant reductions of both R_H (25%) and R_L (12%) in hippocampus. The selectivity of the decline in dentate gyrus implies a degree of specificity of this molecular response to amygdala kindled seizures.

126.8 BINDING OF {^{3}H}-γ-HYDROXYBUTYRATE TO RAT AND HUMAN BRAIN SYNAPTIC MEMBRANES. O. C. Snead, L. H. Bearden, and C. C. Liu. Department of Pediatrics and The Neuroscience Program, University of Alabama in Birmingham School of Medicine, Birmingham, Alabama 35233.

γ-hydroxybutyric acid (GHB) is a naturally occurring metabolite of γ-aminobutyric acid (GABA) which when given to animals produces seizure activity. These seizures are petit mal-like since they are blocked only by anticonvulsants which are specific for their use in petit mal epilepsy (Snead, Neurology 28:643, 1978). GHB has been previously categorized as a GABA agonist by some (Meldrum et al, Brain Res. Bull 5 (Suppl. 2): 685, 1980) and its epileptogenic effects thus attributed to "GABAergic" actions in spite of the fact that it has no affinity for the GABA receptor (Enna and Maggi, Life Sci. 24, 1977). We have thus sought a specific binding site for GHB in rat and human brain as an alternative explanation for the epileptogenic effects of this drug.

{^{3}H}-GHB (20.2|Ci mMol) was synthesized by tritiation of γ-crotonolactone and hydrolyzing the resulting {^{3}H}-GBL. Thin layer chromatography showed that this radioisotope was isographic in 3 different solvent systems.

Synaptic plasma membranes were isolated from both human and rodent brain by standard fractionation techniques in buffered sucrose medium at 3^{0}C. Membrane binding experiments were carried out via a centrifugation assay in a medium containing 50 mM pipes buffer, pH6.5; 1.2 mM Mg^{++}, 2.5 mM Ca^{++}, 12 mM Cl^- and 50 mM K^+ at 4^{0}C. We observed a binding site in both human and rodent brain with a K_d of 4 X 10^{-6}M and B_{max} of 39 pmole/mg protein. The binding was saturable and reversible and the affinity for {^{3}H}-GHB decreased with increasing pH above 6.5. Competition experiments showed that {^{3}H}-GHB binding was not displaced by GABA, γ-butyrolactone, ethosuximide, trimethadione, or valproic acid.

These findings provide evidence for a specific, GHB sensitive, GABA-insensitive receptor binding site in brain that could conceivably mediate the epileptogenic effects of GHB and raise the possibility that GHB may function as a biologically significant substance in brain independent of GABA.

126.9 LONG TERM REDUCTION IN BETA-ADRENERGIC RECEPTOR BINDING FOLLOWING AMYGDALA KINDLING. D.C. McIntyre*, D.C.S. Roberts, D. Milstone*, N. Edson* and L.Bigras* (SPON: J.B.Kelly). Dept. of Psychology, Carleton Univ., Ottawa, Ontario, CANADA K1S 5B6.

Norepinephrine normally provides considerable inhibition against the development of kindled seizures. This influence seems to be primarily through beta-adrenergic transmission. It has been suggested that kindling may be the result of a progressive erosion of this inhibition through down-regulation of the beta-adrenergic sites (McIntyre, Kindling Two, Raven Press, 1981). To test this hypothesis, male Wistar rats in three groups were implanted with bipolar electrodes in both amygdalae. In two of the groups, kindling was precipitated by the daily application of a 2 sec, 60 Hz, 50 uA (peak to peak) sine wave stimulus to the right amygdala, while the third group served as the operated control. Rats in one of the two kindled groups received six stage-5 generalized convulsions, one each day, and then were allowed 23 days rest until sacrifice. Rats in the other group received only five convulsions prior to a 22 day rest, but then received their sixth convulsion 24 hr before sacrifice. All rats, including controls, experienced similar handling. Following decapitation, blunt dissection over ice and storage at -70 C, brain tissues were assessed for dihydroalprenolol (DHA) binding by the method of Bylund and Snyder (Mol. Pharmacol.12:568,1976).

Analysis of three discrete forebrain structures (amygdala, hippocampus and anterior neocortex) indicated significant reductions in 3H-DHA binding in those animals receiving six convulsions 23 days before sacrifice, compared to operated controls, while those receiving a convulsion 24 hours before sacrifice exhibited intermediate values which were not different from either of the other two groups. Scatchard analysis revealed no difference between the groups in binding affinity.

Amygdala kindling seems to result in a significant reduction in beta-adrenergic binding more than three weeks after the last convulsion which, paradoxically, can be partially ameliorated by a single convulsion 24 hours prior to sacrifice. These results suggest that a permanent reduction in the inhibitory influence of the beta-adrenergic mechanism may underlie kindling. (Supported by the NSERC and MRC grants to D.C.M. and D.C.S.R.).

126.10 LONG LASTING CHANGES IN NORADRENALINE METABOLITES FOLLOWING AMYGDALA KINDLING IN RATS. M.M. Okazaki*, J.J. Warsh and W.M. Burnham. Dept. of Pharmacology, University of Toronto and Section of Biochemical Psychiatry, Clarke Institute of Psychiatry, Toronto, Canada.

Adult, male Royal Victoria hooded rats were implanted with bipolar electrodes in the right amygdala, and stimulated once daily until six Stage 4-5 "kindled" seizures had been elicited. Yoked, implanted control rats received identical handling but no stimulation. Two months after the completion of kindling, both kindled and control rats were sacrificed by decapitation, their brains were dissected into 13 regions, and measurements were made of the levels of two major noradrenaline metabolites: 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) and 3,4-dihydroxyphenylethyleneglycol (DHPG). Total MHPG and DHPG levels were simultaneously measured by gas chromatography-mass fragmentography using the protocol of Warsh et al (J. Neurochem. 36:893-901, 1981). It was found that MHPG levels were significantly decreased in the right amygdala (85.3% of control), right hippocampus (91.5% of control), hypothalamus (90.5% of control) and left cortex (95.2% of control). DHPG levels were significantly decreased in the right amygdala only (80.3% of control). No changes were found in left amygdala, left hippocampus, right and left basal ganglia, right cortex, thalamus, midbrain, brainstem or cerebellum. The data suggest that amygdala kindling produces long lasting decreases in noradrenergic neuronal activity in several brain regions.

126.11 MEDIAL SEPTUM KINDLING OF RAT HIPPOCAMPUS PRODUCES NO LASTING RECEPTOR CHANGES. A. M. Morin* and C. G. Wasterlain*. (SPON: J. Engel). Epilepsy and Neurology Res. Lab., V.A. Medical Center, Sepulveda, CA 91343 and Dept. of Neurology, School of Medicine, University of California at Los Angeles.

The kindling model has been used as a tool to study the development and expression of seizures. Changes in muscarinic cholinergic receptors have been reported in electrical kindling of the amygdala (Fitz and McNamara, Brain Res., 178:117, 1979). Septal kindling has several advantages for the study of these changes: a large monosynaptic muscarinic projection from medial septum through the fimbria is the source of over 90% of muscarinic synapses in hippocampus; every medial septal stimulation elicits a prominent hippocampal afterdischarge; and section of the fimbria abolishes kindled seizures elicited from medial septum. Thus medial septal stimulation provides a monosynaptic input into hippocampal muscarinic receptors. In the present study, we have measured both muscarinic and benzodiazepine receptor binding in septal kindled rats. The areas studied are the hippocampus and the cortex. Sprague-Dawley rats (300 gm) were implanted with electrodes in the medial septum and rested. After 1 week, animals received 3 times daily 400 uamp for 1 sec duration. When animals had 3-5 stage 5 seizures they were either killed by decapitation at 30 minutes (acute) or killed one week later (rested). Brains were removed from decapitated animals and various brain areas dissected. Tissue samples were homogenized in 0.32M Sucrose, centrifuged at 1000Xg for 10 min and the supernatants recentrifuged at 50000Xg for 10 min. Resuspended pellets were assayed for binding of ^{3}H-diazepam and ^{3}H-quinuclidinylbenzilate to the benzodiazepine and muscarinic receptors respectively. Left and right hippocampi and left and right cortex samples were measured independently and averaged together for each animal. Values obtained for specific ^{3}H-(-)-QNB (0.12nM) binding in hippocampus were 798.6±93.6 (n=10) controls; 766.9±62, n=8 Acute; and 802.9±105.6 n=8 rested. Values for ^{3}H-(-)QNB binding in cortex were 885.3± 52.9 n=10 control; 901.8±117.2 n=8 Acute; 914.6±74.6 n=8 Rested. There were no signnificant differences in muscarinic receptor binding among control, acute or rested animal tissues homogenates in this type of kindling. Values obtained for specific ^{3}H-diazepam binding in hippocampi were 357.7 ±39.7 fmoles/mg, n=10 Control; 363.2±63.5, n=8 Acute; 402.6±60.9 n=8, Rested. In extensively washed membranes and in whole cortex homogenates, there were no observable differences in GABA (10μM) stimulation of benzodiazepine binding. Based on these observations and our previous studies on electrical and chemical kindling in the amygdala and medial septum, we suggest that receptor response to the kindled seizure may be very subtle or transient and not easily measurable with radioligand binding assays.

126.12 INHIBITED MITOCHONDRIAL CALCIUM TRANSPORT - A MODEL FOR STUDYING EPILEPTIC SEIZURES.

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The requirement of Ca^{2t} for the release of neurotransmitter substances from the synoptic vesicles of nerve endings has been well documented. If calcium influx into the presynaptic endings is a function of depolarization, and if too the calcuim concentration must fall in order to stop the release of nuerotransmitters, there must be mechanisms in the presynoptic cytoplasm which contribute to the decrease in Ca2t. Mitochondrial Ca2t sequestration, endoplasmic sequestration or release to the extracellular space. Our studies with ruthineum red (R.R.) a specific inhibitor of mitochondrial Ca^{2t} transport (Moore, C.L., Biochem., Biophys. Rsch. Commun. 42: 298, 1971) that mitochondria located in the presynaptic cytoplasm could be indicted. Stereotaxic injections of nanomolar amounts of R.R. into several regions of the motor cortex and lateral ventricles, caused mild to severe seizure activity. The type and degree of seizure activity is now quite predictable.

Electronmicroscopy and light microscopy revealed the needle track and the dissipation of the dye, could be working in the cytoplasmic but it would only be blocking the exit of calcium. Mitochondria isolated from brain slices which were incubated with RR or from RR treated synaptosomes are unable to accumulate Ca^{2t}. We therefore propose that one mechanism which may be responsible for seizure activities in the CNS, could be the inability of the mitochondria of the presynaptic cytoplasm to accumulate Ca^{2t}, either because of energy considerations, or a poor Ca^{2t} transport system.

126.13 IMMUNOHISTOCHEMICAL LOSS OF CALCIUM-BINDING PROTEIN FROM DENTATE GRANULE CELLS DURING KINDLING-INDUCED EPILEPSY. K.G. Baimbridge* & J.J. Miller. Dept. of Physiology, Univ. of British Columbia, Vancouver, Canada V6T 1W5.

In a previous study concerned with an examination of biochemical correlates of kindling-induced epilepsy, we reported a specific and long-term decrease in the level of hippocampal calcium-binding protein (CaBP) following kindling stimulation applied to the commissural pathway (Miller & Baimbridge, 1981). In view of these data and the fact that CaBP is localized to CA1 pyramidal and dentate granule cells of the hippocampal formation, the present investigation was undertaken to determine: 1) the localization of this decrease in the hippocampus using immunohistochemical procedures; 2) whether there is a progressive change in CaBP levels during the kindling process or whether the observed decrease results from the seizure activity per se; 3) if the decrease in CaBP reflects a decrease in calcium binding capacity.

Immunohistochemical examination of the hippocampal formation of commissural kindled rats revealed a marked loss of CaBP-like immunoreactivity in the molecular and granule cell layers of the dentate gyrus as well as in the mossy fiber projection to the CA3 region. There was no apparent decrease in the staining of CA1/CA2 pyramidal neurones or in other extra-hippocampal regions.

The concentration of CaBP in the hippocampal formation of animals at different stages of kindling was determined by a specific radioimmunoassay. The results indicated a progressive decrease in CaBP which corresponded closely to the different stages of kindling and the numbers of stimulation trials: Control 994±23 ng/mg total soluble protein; Stages 1-2, 10 trials= 829±9 ng/mg TSP; Stages 3-4, 20 trials=745±14 ng/mg TSP; Stage 5, 30+ trials=667±15 ng/mg TSP. Confirmation that the decrease in CaBP precedes full motor seizure was shown by the fact that repeated metrazol-induced motor seizures (10-14 over a period of 18-21 days) did not significantly alter hippocampal CaBP levels. Additionally, equilibrium dialysis experiments have shown a loss of high-affinity calcium binding capacity in the hippocampal formation of kindled rats suggesting that the observed decrease in immunoreactivity reflects a loss of CaBP function, namely the ability to bind calcium with high affinity.

These data indicate that a specific biochemical correlate of kindling-induced epilepsy is the loss of both CaBP and high-affinity calcium binding capacity within the dentate granule cells and their processes and further that this decrease precedes the onset of full motor seizures. Functionally, this may represent a progressive impairment in calcium-buffering capacity during the process of epileptogenesis.

126.14 SYNAPTIC MEMBRANE PROTEINS IN THE KINDLING MODEL OF EPILEPSY: CALCIUM-CALMODULIN STIMULATED PHOSPHORYLATION AND REGIONAL VARIATIONS. C. G. Wasterlain* and D. B. Farber (SPON: I. Gerson). VA Medical Center, Sepulveda, CA 91343, Dept. of Neurology and Jules Stein Eye Institute, UCLA Sch. of Med.

Septal kindling is associated with changes in the in vitro phosphorylation of hippocampal synaptic plasma membrane proteins (Trans. Am. Soc. Neurochem. 13:83, 1982). We investigated the effects of second messengers, ACTH and phenytoin on ^{32}P incorporation into those specific proteins. In controls, calcium (0.1mM) plus calmodulin markedly stimulated the incorporation of ^{32}P into synaptic plasma membrane proteins of approximate molecular weight 50K, a 58-60K doublet, 62K, 80K, and 3 proteins higher than 100K; and inhibited the phosphorylation of a protein of apparent molecular weight 52K. Stimulation of ^{32}P incorporation into the 50K, 58 and 60K proteins was much less effective in hippocampal synaptic plasma membranes of kindled animals. Calcium, cAMP or cGMP modulated the phosphorylation of several synaptic plasma membrane proteins in a similar fashion in both control and kindled rats.

Calmodulin alone had no effect. ACTH (1-30 units) inhibited in a dose-dependent manner the calcium + calmodulin-stimulated phosphorylation of several proteins including the 50K. Phenytoin similarly inhibited the calcium+calmodulin stimulation of ^{32}P incorporation into several proteins including the 58-60K doublets. The 50 K phosphoprotein was most abundant in hippocampus and in a region including entorhinal cortex and amygdaloid nuclei. It was present in cortex, basal ganglia and brainstem, but minimal concentrations were found in cerebellum and spinal cord. Differences between control and kindled animals in calcium + calmodulin-stimulated phosphorylation of the 50K and 58-60K proteins were most prominent in hippocampus, absent in cerebellum and spinal cord. The possible identity of the 50K protein with B_{50} of Zwiers et al., (J. Neurochem. 33:247, 1979) and of the 58-60K proteins with DPH-M and DPH-L of DeLorenzo (Biochem. Biophys. Res. Com. 71:590, 1976) is under investigation. These data indicate that kindling modifies the response of some synaptic plasma membrane proteins to calcium + calmodulin as tested in vitro. Furthermore, the calcium + calmodulin stimulation of phosphorylation of some of those proteins is most extensive in synaptic plasma membrane from the regions most susceptible to kindling, and nearly absent in regions incapable of it. Supported by the Research Service of the Veterans Administration and by RCDA 5K04 EY 00144.

126.15 INCREASES IN BRAIN THYROTROPIN-RELEASING HORMONE (TRH) FOLLOWING KINDLED SEIZURES. J.L. Meyerhoff, V.E. Bates and M.J. Kubek Dept. Med. Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012, and Dept. of Anatomy, Indiana University School of Medicine, Indianapolis, Indiana, 46223.

Neuropharmacological effects of TRH include enhancement of locomotor activity and antagonism of pentobarbital-induced narcosis. Neurophysiological and neurochemical evidence suggests that TRH may act as a neurotransmitter or neuromodulator in the CNS in addition to its neuroendocrine function. In view of these observations and our recent findings that electroconvulsive shock (ECS) can induce TRH elevations in specific CNS loci, we sought to examine the effects of kindled seizures on TRH in the rat. Kindling consists of daily, low-intensity stimulation of the amygdala which initially produces no effect, but which produces progressively increasing changes in electrical activity and behavior over several weeks, and which finally produces generalized seizures in response to stimulation. Twisted bipolar electrodes were constructed from enamel coated nichrome wire, 125 microns in diameter. These were implanted bilaterally in the amygdalae of male, Sprague Dawley rats. Rats (245 ± 15 g at surgery) were allowed to recover for a week. Experimental animals were stimulated once daily with biphasic square wave pulses to the left amygdala (250 μA, 60 Hz, 1.0 s). Controls were treated similarly with the exception that no current was given. Stimulated animals were considered to be kindled after responding with stage 5 (generalized) seizures on 3 consecutive days. This criterion was reached within 18 ± 2 days. Once kindled, experimental animals were given one additional stage 5 seizure (controls, sham stim.) 48 hrs prior to decapitation. Brains were immediately dissected and frozen on dry ice. TRH was assayed in extracts by specific RIA and results expressed as pg/mg protein (mean ± SEM). Kindling induced marked increases of TRH in the amygdala (196.9 ± 18.9 vs 596.1 ± 102.0, $p<0.005$), hippocampus (44.1 ± 7.5 vs 130.1 ± 17.3, $p<0.005$), nuc. accumbens (154.13 ± 21.3 vs 233.55 ± 24.0, $p<0.05$), and a 4-fold increase in cortex (11.37 ± 2.5 vs 45.98 ± 8.2, $p<0.005$). No significant changes were observed in the corpus striatum, thalamus, midbrain, brainstem, cerebellum, hypothalamus, or pituitary. These results indicate: (1) that kindling can induce significant and prolonged elevations of TRH in specific brain regions after stage 5 seizures, and (2) sites of TRH increase occur in regions associated with epileptic foci. Further studies are planned on the possible role of TRH in the development and pathophysiology of seizures. Supported in part by grant RO1-AM-28260.

126.16 MODIFICATION OF KINDLED AMYGDALOID SEIZURES BY CAFFEINE. T. E. Albertson, R. M. Joy and L. G. Stark. Health Sciences Neurotoxicology Unit, School of Medicine and Veterinary Medicine, University of California, Davis, CA 95616.

Sprague-Dawley rats were chronically implanted with cortical and amygdaloid electrodes. Ten days after surgery, rats were given daily i.p. injections of saline (cc/kg, N=11) or caffeine (50 mg/kg, N=11) twenty minutes before electrical stimulation of the amygdala (biphasic 60 Hz, 1 msec in duration and 400μAMP). Animals were daily injected and stimulated until 3 kindled amygdaloid generalized seizures (KGS) had occurred (rank=5). The animals were then stimulated daily for 3 days without pretreatment followed by 5 more daily caffeine or saline pretreatments and stimulations. There was no significant difference between the two groups in the number of daily stimulations (8.5 saline and 8.2 caffeine) needed to reach the KGS (rank=5) nor was there any significant difference in total cumulative afterdischarge needed to reach the KGS. During the first 7 days of drug treatment, the daily average afterdischarge duration tended to be longer in the caffeine group. This difference became significant (>200% of saline) when the animals reached the KGS stage. When the KGS animals were stimulated without caffeine or saline pretreatment, the daily average afterdischarge duration returned to control group lengths. After reinstitution of saline or caffeine pretreatments, the average afterdischarge durations returned to significantly increased lengths.

Twenty additional fully amygdaloid kindled rats were utilized to evaluate the effects various doses of caffeine (6-50 mg/kg) have on electrically induced suprathreshold (400μAmp) and threshold (20μAmp increments) seizures. Caffeine had no consistent effect on threshold values. Doses of 12-50 mg/kg of caffeine resulted in more severe behavioral ranks and significantly longer afterdischarge durations. With suprathreshold stimulation, the afterdischarge duration was significantly increased only after the highest dose of caffeine. From this data it would appear that caffeine lengthens induced afterdischarges both in fully kindled animals and during the acquisition phase. In the doses tested, caffeine does not appear to significantly reduce the seizure threshold in KGS rats, nor does it appear to increase the rate of acquisition of the KGS. It is postulated that caffeine modification of KGS may be through an inhibition of the mechanism which terminate seizures. (Supported by the Northern California Occupational Health Center and GRS 2507RR5457.)

126.17 FURTHER STUDIES OF THE EFFECTS OF NORADRENALINE DEPLETION ON KINDLING. V. Westerberg*, I. M. Altman*, J. Lewis*, and M. E. Corcoran. Department of Psychology, University of Victoria, Victoria, British Columbia, Canada, V8W 2Y2.

Previous work has indicated that depletion of central noradrenaline (NA) by treatment with 6-hydroxydopamine (6-OHDA) facilitates amygdaloid kindling in rats. Although the rate of kindling was increased, the generalized seizures produced in NA-depleted rats were similar in duration and intensity to those in controls, suggesting that NA regulates seizure development but does not affect established seizures. To test this directly we first kindled intact rats with amygdaloid stimulation and, after the development of generalized seizures, subsequently depleted NA with intracranial injections of 6-OHDA. Rats were kindled with daily application of unilateral electrical stimulation of the amygdala; after 3 generalized seizures had been elicited they received bilateral injections of 6-OHDA or vehicle into the dorsal NA bundle. Although forebrain NA was extensively depleted by 6-OHDA, there were no postinjection differences between the seizures of 6-OHDA-treated rats and controls. This finding confirms previous suggestions that NA apparently does not regulate amygdaloid seizures once they have been kindled.

A second experiment sought to determine whether nonlimbic kindling would also respond to depletion of NA. 6-OHDA-treated rats and vehicle-treated controls received daily application of unilateral electrical stimulation of the frontal cortex. The groups displayed indistinguishable patterns of "focal cortical" seizures, but the rate of subsequent genralization to limbic-type seizures was greatly enhanced in the NA-depleted rats (mean of 10.7 afterdischarges to generalization, as compared to 23.4 in the controls). The generalized seizures of the two groups were, however, similar in duration and intensity. Thus depletion of NA facilitates the kindling of generalized seizures with stimulation of either the frontal cortex or amygdala.

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126.18 DOSE-DEPENDENT PROCONVULSANT AND ANTICONVULSANT PROPERTIES OF XYLAZINE ON KINDLED SEIZURES. R. M. Joy, L. G. Stark, T. E. Albertson and J. F. Bowyer*. Health Sciences Neurotoxicology Unit, Schools of Medicine and Veterinary Medicine, University of California, Davis, California, 95616

Xylazine (Rompun®, Bayer 1470) is a novel chemical substance capable of producing sedation, analgesia and muscle relaxation. It has been proposed that these centrally mediated effects result from the agonistic action of xylazine on alpha$_2$ adrenergic receptors. The degree of sedation is dose-dependent, and the central effects are blocked by yohimbine, a selective alpha$_2$ antagonist.

As the consequences of xylazine administration have been related to a reduction in adrenergic activity within the CNS, modifications in seizure susceptibility and response may also be produced by xylazine. In other situations in which a reduction in adrenergic activity in the central nervous system has been produced, a heightened responsiveness to epileptogenic stimuli has been observed. We have investigated this by determining the effects of xylazine on developing and mature kindled amygdaloid seizures (KAS) in rats. Xylazine is used extensively in veterinary and experimental animal medicine, and any modification in seizure susceptibility has clinical as well as general relevance.

Xylazine (0.3-30 mg/Kg) was administered ip to rats 20 minutes before kindling stimuli were presented. Stimulations were given once daily to measure effects during kindling acquisition. In fully kindled subjects it was administered 20 minutes before determining seizure thresholds, durations and severities.

The effects of xylazine on KAS were polyphasic depending on dose. A dose of 0.3 mg/Kg was proconvulsant. It reduced seizure thresholds and increased KAS duration and severity. It also reduced the time required to kindle from 9.8 $\pm$ 0.9 to 7.9 $\pm$ 0.8 days. Doses between 3-20 mg/Kg were anticonvulsant. They increased seizure thresholds and decreased KAS duration and severity. Time required to kindle was not significantly changed. Doses above 30 mg/Kg evoked spontaneous seizures in 4 of 8 fully kindled subjects.

It is not possible at this time to interpret these effects as being the consequence of alpha$_2$ agonistic action. Modifications of seizure susceptibility did not show a simple monotonic relationship with dose as has been shown for certain other actions of xylazine. These results do suggest, however, that caution should be taken when administering xylazine to epileptic or other seizure prone animals.

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126.19 EFFECT OF COCAINE AND PENTYLENETETRAZOL ON CORTICAL KINDLING. Jeffrey S. Stripling and R. D. Russell*. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Kindling is a form of sensitization in which brain stimulation that initially produces only a localized seizure discharge acquires, through repetition, the ability to trigger widespread seizure activity accompanied by a behavioral convulsion. Recently we reported that convulsions induced by cocaine or lidocaine, but not pentylenetetrazol (PTZ), facilitated kindling of the olfactory bulb, suggesting that local anesthetics may be more potent than other convulsants in producing lasting alterations of that area of the brain (Stripling and Hendricks, Pharmac. Biochem. Behav., 15: 793-798, 1981). In order to assess the generality of this finding, the present experiment examined the effect of drug-induced convulsions on kindling of the neocortex, which differs from kindling of the olfactory bulb and other limbic sites in both the rate of development and the form of the kindled seizures. Male Long-Evans rats experienced three convulsions produced by intravenous infusion of cocaine or PTZ at three-day intervals, or received control infusions of saline. Beginning eight days later the animals were kindled by repeated stimulation of the neocortex. Animals which had been convulsed by either cocaine or PTZ exhibited significantly longer afterdischarges and kindled significantly faster than controls. This is a different pattern of results than was found with kindling of the olfactory bulb, where convulsions induced by PTZ were without significant effect. Thus there appears to be a difference in the anatomical distribution of the effects of cocaine and PTZ on kindling. Following the completion of kindling these drugs were further compared by examining the effect of subconvulsive doses on the expression of fully kindled seizures. The two drugs had contrasting effects on the afterdischarge threshold of kindled neocortex: cocaine elevated the afterdischarge threshold to more than twice its control value, while PTZ resulted in a small decrease in threshold. However, similar effects were seen on the form of the behavioral convulsions elicited by stimulation: both drugs significantly reduced the duration of the initial tonic phase of the convulsion. These post-kindling drug tests reveal both similarities and differences in the effects of cocaine and PTZ on seizure susceptibility. The kindling phase of this experiment, in conjunction with previous research, suggests that the influence of a drug-induced convulsion on subsequent kindling can depend upon both the drug used and the site of stimulation.

126.20 EFFECT OF PROSTAGLANDIN SYNTHETASE INHIBITORS ON CONVULSIVE THRESHOLD IN RATS. M.C. Wallenstein and E.A. Mauss*. New York University, New York, NY 10010.

In spite of the fact that many substances of widely different chemical structure produce seizures, the resulting electrical alterations at the neuronal level tend to be very similar. This suggests a final common pathway. Prostaglandins (PGs) are present in nervous tissue and are released in increased amounts during both spontaneous and experimental seizures. However, whether PGs have a role in induction of convulsions is still unclear. In the present study, the effects of PG synthetase inhibitors on convulsions induced by either flurothyl (inhalation), picrotoxin (2 mg/kg ip) or electroconvulsive shock (150 ma; 0.2 sec) were evaluated. It was found that the effect of pretreatment with PG synthetase inhibitors on flurothyl-induced and electroconvulsive shock-induced convulsions was varied. Ibuprofen, sulindac, mefenamic acid and meclofenamic acid increased the latency to flurothyl-induced convulsions and decreased the intensity of electroconvulsive shock-induced convulsions whereas paracetamol and indomethacin had no significant effect on either type of seizure. Higher doses of either mefenamic acid (150 mg/kg) or meclofenamic acid (150 mg/kg) were themselves convulsant. In contrast, picrotoxin-induced convulsions were not significantly affected by any of the above agents with respect to latency, number of convulsions per animal, duration of individual convulsions or percentage deaths. These results suggest that PGs are involved in the mechanisms underlying flurothyl- and electroconvulsive shock-induced convulsions but not picrotoxin-induced convulsions.

(Supported by grant from The New-Land Foundation)

127.1 CONSUMMATORY BEHAVIOR AND PITUITARY-ADRENAL RESPONSIVENESS IN THE HAMSTER. Joanne Weinberg and Roderick Wong*. Department of Anatomy and Department of Psychology, University of British Columbia, Vancouver, B.C. V6T 1W5.

Data on rats has shown that opportunity to perform a consummatory response can inhibit the activity of the pituitary-adrenal system. In this study we examined the effects of consummatory behavior on adrenocortical activity in the golden hamster (Mesocricetus auratus). Because hamsters are desert rodents, their internal regulatory systems as well as their behavioral responses to food or water deprivation differ markedly from those of the rat. Unlike rats, hamsters appear behaviorally unresponsive to the duration of a fast, and show no increase in either the size or frequency of their meals or their water intake following deprivation. Therefore we were interested in determining whether hamsters which have been water deprived can use the cues or reinforcement provided by consummatory behavior to decrease their adrenocortical response to stress.

Both males and females were included, and all animals were water deprived for 24 hr prior to testing. Testing took place either in a novel cage or in the home cage. During the 30 min test session animals were allowed access to water or remained deprived. Cortisol levels were measured both prior to and immediately following the test session.

We found that: 1) Animals tested in the home cage began drinking faster and drank more water than animals tested in a novel cage. 2) Basal levels of cortisol were not elevated following 24 hr water deprivation. 3) Placement in a novel cage produced significant cortisol elevations in both males and females. However, if water was available, cortisol levels were markedly reduced, and did not differ significantly from presession (basal) levels. 4) In contrast, opportunity to drink in the home cage produced significant cortisol elevations. Thus the act of drinking appears to reduce the arousal level of hamsters tested in a novel environment but to increase the arousal level of animals tested in a familiar setting.

In a second experiment we examined whether length of exposure to a novel environment would affect the elevation in plasma cortisol which was observed. Animals were placed in a novel cage for 5 or 25 min, and blood samples were taken either 30 or 60 min after the start of the test session. Five min exposure produced no elevation in cortisol. After 25 min however, males showed greater cortisol elevations than females.

Supported in part by a grant from NSERC of Canada to R. Wong.

127.2 MATERNAL BEHAVIOR MODULATES PITUITARY-ADRENAL ACTIVITY OF RAT PUPS AFTER EARLY STIMULATION. William P. Smotherman, Department of Psychology, Oregon State University, Corvallis, OR 97331.

Litters of rat pups that receive additional stimulation early in life have been found to differ from control (unstimulated) litters when tested as adults. Different hypotheses have been suggested to explain these findings. One such hypothesis suggests that alterations in maternal behavior following return of stimulated pups to the nest accounts for the observed adult behavior differences. In this study, two-day-old rat pups were handled, exposed to electric shock or left undisturbed in their home cage for a 3-minute period. For half of the litters within each of these treatment conditions the mother was returned to the nest and allowed to engage in maternal behavior. For the remaining litters within each treatment condition the mother was placed behind a wire mesh screen and prevented from interacting with the pups. Litters were sacrificed immediately after their return to the home cage, 20, or 60 minutes later. Blood samples were collected from individual pups and pooled to yield a single sample from a litter. Samples were assayed fluorometrically to determine plasma levels of corticosterone. The 3-factor ANOVA indicated the significant 3-way interaction of Maternal Condition (Present/Absent) by Pup Treatment (Control, Handled, Shock) by Time Points (0, +20 minutes, +60 minutes) $F(4, 126) = 18\text{-}92$, $p<.01$. Post-hoc tests for simple interaction effects indicated the significant Pup Treatment by Time Point interaction in the Mother Absent condition only ($p<.01$). Here, shocked pups not experiencing active maternal care showed greater elevations in corticosterone both 20 and 60 minutes after their return to the home cage following treatment compared to undisturbed control and handled litters ($p's<.05$). These data show that early stimulation triggers pituitary-adrenal activity in the 2-day-old rat pup and further, that the effect of this early stimulation is modulated by the maternal behavior that stimulated pups receive after their return to the nest. These data are consistent with the maternal mediation hypothesis of early experiences. (Supported by NICH and HD grant HD 16102-01).

127.3 EPINEPHRINE ALTERS THE EFFECT OF AMYGDALA STIMULATION ON RETENTION OF AVOIDANCE TASKS. C. Bennett*, K.C. Liang* and J.L. McGaugh (SPON: B.J. Vasquez). Department of Psychobiology, University of California, Irvine, CA 92717, U.S.A.

We previously reported that the amnestic effects of post-training electrical stimulation of the amygdala in rats could be attenuated by prior adrenal demedullation. In the present study we investigated in more detail the contribution of the adrenal medulla to the amnestic effect of amygdala stimulation.

Rats were implanted bilaterally with electrodes aimed at ABM/ABL amygdala. Approximately half were adrenal demedullated (ADMX) and the remainder were sham demedullated (SHAM). Two weeks after the adrenal surgery, they were trained on a one-trial step-through inhibitory avoidance task (1 mA, 2 sec footshock). The rats were then randomly assigned to new groups and trained 14-16 days later on an 8-trial active avoidance task (640 μA, 30 sec footshock). In 8 of the groups injections of epinephrine (E) (1 mg/kg) or saline were given subcutaneously immediately following training. These rats then received amygdala stimulation (AS) (50 μA/electrode, 30 sec) or sham stimulation (IC). In 2 groups, IC/ADMX-d and AS/ADMX-d, the injection of E was delayed for 3 min after the training, and given after the amygdala stimulation. Results of the 24 hr retention tests appear in the table.

We replicated previous findings that the AS/SHAM and IC/ADMX, saline groups showed severe deficits in both tasks. The demedullation, as before, significantly attenuated the deleterious effects of AS in the ADMX rats as compared to rats with intact adrenals. All effects were completely reversed by administration of E: E attenuated the impairment in the IC/ADMX group and reinstated the amnestic effects of amygdala stimulation in the AS/ADMX group. Further, the effect of E replacement was highly time-dependent in the AS/ADMX group. If the E injection was delayed until after the amygdala stimulation, the stimulation did not produce amnesia.

We conclude that adrenal release of E after training contributes to subsequent good retention performance. This effect is revealed in the ADMX group of animals having also the small lesion effect of the implant (IC). Exogenous E can reverse this deficit. Additionally, the data suggest that endogenous E released from the adrenals after footshock must interact with the amygdala stimulation for the amygdala stimulation to be an effective amnestic treatment.

		SHAM/0	SHAM/1.0	ADMX/0	ADMX/1.0	ADMX/1.0-d
Inhibitory (median-sec)	IC	549.1	575.7	164.0	475.5	341.3
	AS	98.5	55.9	245.5	86.7	325.4
Active (Day 2-1) ($\bar{x}$ # of avoids)	IC	2.94	3.40	0.87	3.29	2.50
	AS	1.38	0.90	3.00	1.07	2.46

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127.4 PITUITARY CYCLIC AMP IN RATS: EFFECTS OF PRESENTING OR WITHHOLDING AN APPETITIVE STIMULUS. B.N. Bunnell, J.L. Meyerhoff, D.R. Collins*, E.H. Mougey*, L.L. Pennington*, and R.H. Lenox. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012.

Cyclic AMP appears to be involved in the release or synthesis of pituitary hormones mediating the organism's response to stress since both physical and psychological stressors have been shown to increase pituitary cyclic AMP (Neurosci. Abst. 108.17 and 282.2, 1981). This study was undertaken to determine if such increases might also occur after exposure to appetitive stimuli. To eliminate the possibility that food deprivation might produce stress in the animals, we utilized rats' predatory responses to crickets. Most rats, when presented with crickets, will kill and eat these insects with a very short latency and without being food deprived. Eighteen male SD rats, adapted to handling for one week, were maintained on ad libitum food and water. During an habituation period 12 rats were given daily opportunities to kill and eat a cricket. On the test day, 6 rats were sacrificed 5 minutes after beginning to eat a cricket (Group A), the other 6 habituated rats were placed in the test cage but not given a cricket (Group B), while the remaining 6 animals were taken from their home cages and sacrificed immediately (Group C). All rats were sacrificed by exposure to high-intensity microwave irradiation (5.0 sec., 2.5 KW, 2450 MHz). Pituitary(PIT) and hypothalamic(HY) cyclic AMP were measured by radioimmunoassay. The results are given in pmoles cyclic AMP/mg wet weight ± S.E.M..

	CYCLIC AMP	
	Pituitary	Hypothalamus
Group A	1.27 ± .10	0.83 ± .08
Group B	1.82 ± .15	0.94 ± .07
Group C	1.41 ± .10	0.87 ± .10

The rats in the group that killed and ate crickets on the test day (Group A) did not have an elevation in pituitary cyclic AMP. A small, but statistically significant increase in pituitary cyclic AMP was seen in the rats from which crickets were withheld (Group B). This is consistent with our previous findings that stress increases pituitary cyclic AMP, as these rats may have been stressed by the withholding of an expected appetitive stimulus.

127.5 PLASMA CORTICOSTERONE DECREASES PRODUCED BY EXPECTANCY OF LARGE TOTAL FOOD REWARD IN OPERANT TASKS. G. Coover, P. Senkowski* and P. Colsher*. Dept. of Psychology, Northern Illinois Univ., DeKalb, IL 60115.

Rats exhibit a rapid decline in plasma concentration of corticosterone when fed their once-daily meal (e.g., Coover et al., Soc. Neurosci. Abstr., 6:172, 1978). However, the half-dozen studies in the literature which examined corticosterone changes during sessions of lever pressing for food or water reward have reported either maintained or increased hormonal levels during such sessions. This discrepancy in findings between home-cage feeding and instrumental training situations suggests the possibility that aspects of the work situation may produce stress which attenuates, or precludes, declines in corticosterone level.

The present experiment monitored the instrumental behavior and corticosterone levels of eight groups of rats which received extensive training on variable interval (VI) 24-sec schedules. Each reinforcement consisted of two .045-g food pellets. Three task characteristics were varied in a 2X2X2 factorial design: session duration (15 or 30 min), type of instrumental response (lever press or entry into a recessed food cup), and type of schedule construction (an arithmetic or constant probability series of intervals). On blood-sampling days, all sessions were 15 min. The clearest effect was that of session duration. Subjects normally given 30-min sessions, and thus "expecting" a total of approximately 6 g of food, exhibited relatively greater declines in plasma corticosterone than did subjects normally receiving 15-min sessions (total expected reward = 3 g). Thus, rats can exhibit an anticipatory decline in corticosterone during instrumentally reinforced training sessions if they have normally received a sufficiently large total amount of reward.

The presence of higher-order interaction effects indicated that corticosterone levels were also influenced by the instrumental response required and the method of construction of the VI schedule. Although the instrumental response requirement had no reliable effect on the corticosterone levels of subjects trained using the arithmetic schedule, those of subjects trained using the constant probability schedule were lower among lever-press as compared to goal-entry subjects. Further specification of the situational variables in instrumentally reinforced tasks which affect the pituitary-adrenal system may greatly increase the utility of such tasks in the examination of other systems. Such research could also suggest factors which may contribute to stress in the work environment.

127.6 THE EFFECT OF ADRENALECTOMY AND HYPOPHYSECTOMY ON NEUROTENSIN-INDUCED CYTOPROTECTION AGAINST STRESS-INDUCED GASTRIC ULCERS IN RATS. D.E. Hernandez, J.W. Adcock*, C.B. Nemeroff, R.C. Orlando* and A.J. Prange, Jr. Biological Sciences Research Center, Departments of Psychiatry and Medicine, University of North Carolina School of Medicine, Chapel Hill, NC 27514.

Previous studies have demonstrated that intracisternal (IC), but not peripheral (IV), administration of neurotensin (NT), an endogenous tridecapeptide, significantly reduces the incidence of gastric ulcers in a dose-dependent manner in a standard model of cold-restraint stress (CRS) in rats (Amer. J. of Physiol., in press, 1982). This "cytoprotective" effect appears to be unrelated to NT-induced changes in body temperature or to NT's neuroleptic-like effects. Moreover, NT does not inhibit gastric acid secretion. NT's protection against CRS ulcers appears not to be mediated by an effect on pituitary hormone secretion. It does, however, require an intact prostaglandin synthetic pathway.

We have suggested that the adrenergic division of the autonomic nervous system (ANS) plays an intermediary role in NT-induced gastric cytoprotection (Ann New York Acad. of Sci. 1982, in press). The purpose of the present report was to evaluate the effect of hypophysectomy and adrenalectomy on the cytoprotection produced by IC NT in rats exposed to CRS.

Intact, hypophysectomized or adrenalectomized, and sham-operated adult males S-D rats (200-250g) were food--but not water --deprived, for 24 hr before experiments. Then rats were lightly anesthetized with ether and injected IC (10 µl) with either vehicle (0.9% NaCl) or NT (30 µg). Immediately after the injections all rats were restrained in wire mesh and placed supine in a cold room (4°C) for 3 hr. Rats were then killed by decapitation and the stomachs examined for gastric ulcerations by a trained observer unaware of treatments.

In confirmation of previous studies NT (30 µg IC) produced a significant reduction in the incidence of gastric ulcers. Adrenalactomy, but not hypophysectomy, totally abolished the gastric cytoprotective effect of NT. In the hypophysectomized rats NT produced a significant cytoprotection 5 days, but not 2 weeks after surgery, suggesting that the trophic effect exerted by the pituitary on the gastric mucosa is not an essential requirement for the acute prevention of stress ulcers by NT.

These results, taken together with previous findings are congruent with the view that the sympatho-adrenal axis may play an intermediary role in NT-induced cytoprotection in this CRS model.

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127.7 EFFECTS OF ADRENAL STEROIDS AND THEIR REDUCED DERIVATIVES ON CNS EXCITABILITY. B. Dubrovsky*, I. Kraulis* and D. Williams*. (Spon: R. Malmo). Allan Memorial Institute, McGill University, Montreal, Quebec, H3A 1A1.

We have shown (Brain Res. 88, 1, 1975) that labelled DOC concentrates preferentially in brainstem regions of the reticular formation (RF). The amplitude of sciatic evoked potentials (EP) in pontine RF was significantly decreased after acute DOC treatment. Our data also showed that the high concentration of brainstem radioactivity may be accounted for by a less polar metabolite of DOC with the chromotographic characteristics of 3-∝OH 5∝ tetrahydro-DOC (THDOC). The structure of this metabolite resembles those of known steroid anaesthetics. We decided then to study the effects of reduced derivatives on CNS excitability. In adrenalectomized rats, under urethane anaesthesia, IV injection of DOC, 5∝dihydro-DOC, or THDOC, 750 µg in .5 ml of 4:1 saline: cremophor-EL solution, produced a significant decrease in the amplitude of sciatic EPs recorded in pontine RF regions. The onset and duration of the steroid effect was shorter for the reduced metabolites than their parent compound. Further, we studied the effects of these steroids on tonically firing neurons in brainstem regions. The effects of DOC were studied on 35 neurons of which 18 responded with a decrease in their firing rate. In all cases where the neurons were responsive to DOC, the distribution of the interval histograms showed a decrease in the peak of the shorter intervals and a broader distribution extending towards longer intervals. Ten cells were tested with THDOC and responded in a similar fashion. Control injection of the vehicle had no effect. In all cases the onset of action was between 1-5 minutes and the observed effect generally lasted 20 minutes. We then studied the effects of corticosterone (B), and its ring A reduced metabolite 5∝dihydro-B. Out of 40 neurons tested with B, 16 responded with a significant increase in their mean firing frequency. In contrast, injection of 5∝dihydro-B induced a significant decrease in the firing rate of 12 of 16 neurons studied. Similarly, EP studies revealed enhanced responsiveness with B and decreased responsiveness with dihydro-B. The effect of consecutive injection of the reduced and parent compound was studied in 8 neurons. While all neurons responded to 5∝dihydro-B with a decrease in their firing rate, injection of B reinstated preinjection firing levels in 4 neurons. Recently we showed that 18OHDOC, a major adrenal steroid in the rat, significantly decreased responsiveness in pontine RF regions to sciatic stimulation (Endoc. Soc. Meet. 1982). In conclusion, our studies indicate that adrenal steroids, as well as their ring A reduced metabolites, may fulfill an important biological role in modulating activity of certain CNS regions. Supported by the George C. Stairs Foundation.

127.8 STRIATAL DOPAMINE-STIMULATED ADENYLATE CYCLASE MAY CORRELATE WITH MAMMARY TUMOR GROWTH. P.R. Goldman* and W.H. Vogel, Dept. of Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107.

An inverse correlation has been reported between the striatal dopamine-stimulated adenylate cyclase (DAC) activities of female mice from various strains and their incidence of breast cancer. (Sci. 197, 1094 (1977)). We investigated a possible role of DAC activity in the growth of 7,12-dimethylbenz(∝)anthracene (DMBA) induced tumors in different strains of rats and in the inhibition of this growth by immobilization stress in Sprague-Dawley (SD) rats. Female rats from three strains, SD, Wistar (W) and Long Evans (LE) were used for DMBA tumor induction. Five weekly doses of 5 mg per rat were given. The mean tumor weights obtained after 10 weeks were SD: 5.1±11.7 g, W: 9.5±17.8 g and LE: 0.5±1.6 g. The SD rats did not differ significantly from the W rats but both had significantly higher tumor weights than LE rats. The mean DAC activities in parallel groups of these strains were also measured, SD: 136.8±21.3%, W: 130.8±28.4% and LE: 181.0±63.9%. SD rats were not significantly different from either W or LE rats. However, W rats differed significantly from LE rats, $p < 0.025$. Two substrains of SD rats which differ in their susceptibility to DMBA tumor induction also had significantly different DAC activities. The substrain with the lower tumor weight had a mean DAC activity of 143.1±31.3% whereas the more susceptible substrain had a mean DAC activity of 113.0±28.1%. Short term immobilization stress was not found to influence the DAC activity whereas long term immobilization which decreases tumor weight significantly increased DAC activity from 109.4±12.1% in control animals to 128.6±20.8% in animals which were chronically immobilized. These data show that an inverse relationship exists between striatal DAC activity and DMBA tumor growth in rats.

127.9 PRENATAL STRESS EFFECT REVISITED: PITUITARY-ADRENAL MEDIATION. Sonya K. Sobrian and Kim E. Armstrong*. Depts. of Pharmacology and Psychology, Howard Univ., Col. of Med., Washington, DC 20059.

Previous research has indicated that in rodents a variety of prenatal stressors can alter both the developing and adult behavior of the offspring. To test the hypothesis that these effects are mediated by the maternal and/or fetal pituitary-adrenocortical axis (PAC), we determined if pharmacological activation or suppression of the PAC would mimic the effects of prenatal environmental stress on behavioral maturation previously reported by our laboratory.

Timed-pregnant Wistar rats were injected s.c. on gestation days 14-20 with either ACTH (14 I.U.), corticosterone (CORT: 7.5 mg/kg), dexamethasone (DEX: 150µg/kg), or saline (SAL: 0.1ml/100g). Pups were delivered naturally and cross-fostered at birth (Day 1) to non-treated females. Physical,reflex and behavioral development were monitored every 3-5 days from birth to 30 days of age. Maternal and neonatal plasma corticosterone levels were measured at birth, and at 15 and 30 days of age in the offspring.

Gestational length, litter size, weight and length were unaffected by prenatal drug treatment; in contrast the male-female ratio in both the ACTH (2:1) and DEX (1:3) litters were significant different from SAL (1:1). Moreover, pups from both CORT and DEX litters exhibited accelerated pinna elevation, eye opening and development of the righting reflex. Offspring of ACTH females were similar to controls. In a test of olfactory development both CORT and DEX offspring spent significantly less time over home cage shavings; latency to respond to the olfactory stimilus was longer in the DEX pups, but shorter in the CORT pups. The accelerated development of spontaneous motor activity (SMA), previously reported following exposure to prenatal environmental stress, was evident in the corticosterone treated pups. DEX significantly delayed the development of SMA, while ACTH and SAL pups did not differ.

Plasma corticosterone levels and adrenal weight data suggested that alterations in the maternal rather than the fetal/neonatal PAC may mediate prenatal stress effects. Although both DEX and CORT altered behavioral development in the offspring,treatment with CORT mimiced the accelerated development observed following prenatal environmental stress. Treatment with CORT induces maternal adrenal atropy and suppresses adrenal cortical output; endogenous steroid levels are low, while exogenous levels are high. These findings suggest that altered adrenal activity may be responsible for observed prenatal stress effects in the offspring.

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127.10 PLASMA CORTISOL AS A PREDICTOR OF INDIVIDUAL DEPRESSIVE BEHAVIOR IN RHESUS MONKEYS (MACACA MULATTA). J. D. Higley,* S. J. Suomi,* J. M. Scanlon* and W. T. McKinney. Primate Lab, Univ. of Wisconsin, Madison, WI 53706.

Separation from a social group has been shown to produce a biphasic protest-despair syndrome in a number of primate species. Recent studies have indicated a relationship between plasma cortisol and this syndrome. For example, studies have demonstrated a significant early rise in cortisol that peaks within hours and thereafter tapers off but remains above baseline levels; however, there is considerable individual variability in how high this initial peak is (Gunner et al., 1981, Psychoneuroendocrinology, 6). Few studies have investigated the relationship between this variability in early cortisol peak and individual behavior. Based on a previous pilot study, we hypothesized that we could predict which monkeys were most prone to depressive symptoms after separation based on 2-hour cortisol peaks. Specifically, we believed that the higher an infant's plasma cortisol level 2 hours postseparation, the greater the likelihood of depressive behavior. Furthermore, we hypothesized that low cortisol rises during the early 2-hour peak would predict increased protest, but little despair.

We tested 47 rhesus monkeys (23 female, 24 male) ranging in age from 2-7 years. All subjects were living in groups. Each subject was separated from its group for approximately 2 1/2 hours and put in a single cage in a room by itself. Blood was taken either femorally or from the saphenous vein immediately after removing the subject from its group and 2 hours later. Separation behavior was recorded on a videotape and scored on clocks and counters. Based on plasma cortisol peaks, measured in blood plasma by radioimmumoassay, we divided the subjects into three response groups: high responders, medium responders, and low responders.

High responders demonstrated significantly more passive withdrawn depressionogenic behavior such as sitting passively, huddling, self-mouthing and self-clasping. Low responders demonstrated a borderline significant increased protest phase (prolonged locomotion and movement) relative to the other response groups. This is constant with other studies that have demonstrated a depression-adrenal cortisol relationship.

127.11 STRESS AND HEREDITY IN ADRENOCORTICAL RESPONSE IN RHESUS MONKEYS (MACACA MULATTA). J. M. Scanlan,* S. J. Suomi,* J. D. Higley* and G. Kraemer. Primate Lab, Univ. of Wisconsin, Madison, WI 53706.

Previous studies (Schlesser, et al., 1980, Arch. Gen. Psych. July; Higley et al., 1982) have demonstrated positive correlations between cortisol and depressive symptoms in both humans and nonhuman primates. The rhesus adrenocortical responses have a large genetic component.

47 rhesus monkeys were withdrawn from their normal social environment and placed alone in a cage in an unfamiliar, isolated room for two hours. Blood draws were taken before (basal) and after (peak) the two hour period. Because of the social nature of rhesus, we hypothesized that the stress of separation and handling would reliably elicit plasma cortisol increase and symptoms of agitation and depression. This prediction was confirmed, as detailed in Higley et al., 1982.

Heritability was tested by comparison of paternal 1/2 sibs. Comparison was restricted to 1/2 sibs between the ages of 1 year 5 months and 2 years 9 months because of evidence that puberty differentially affected male and female peak cortisol response. Both males and females were pooled, and eleven half sib comparisons were made, yielding a 188 narrow sense heritability for peak cortisol response to the stress of separation.

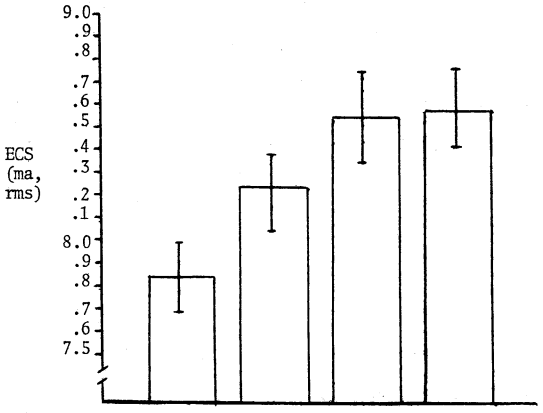
127.12 RESPONSES OF DAIRY COWS TO NON-AVOIDABLE SHOCK DURING MILKING. A. M. Lefcourt, R. M. Akers*, and R. H. Miller*. Milk Secretion and Mastitis Lab, USDA-ARS, Beltsville, MD 20705.

Electrical shock is presumed to be a severe problem on many dairy farms. To determine the relationship between shock and inhibition of the milk-ejection reflex, 15 Holstein cows were accustomed to being milked in an experimental milking parlor for 7 days. Cows were milked in pairs starting at 800 and at 2000 h. The experiment was divided into pre-stimulus (7 days), stimulus (7 days), and post-stimulus (5 days) periods. Initially 3 groups of 5 cows each were to be shocked using 3, 5, or 10 ma (60 Hz) via electrocardiogram electrodes attached to shaved areas above the right front and rear hocks. Shock was administered intermittently (on 5 s, off 25 s). Cows were brought into the parlor, heart rate (HR) was monitored under baseline conditions for 1 min, the udder was washed and dried for 1 min, and then the milking machine was attached. During the stimulus period, the shocker was turned on at the beginning of udder preparation and remained on for 11 min. Milk flow rates were measured at 15 s intervals using a venturi meter. The reaction of cows subjected to 10 ma was so severe that they physically could not be milked. The protocol was altered so that 8 and 7 cows received 5 and 3 ma, respectively. Two additional cows could not be milked at 5 ma and were dropped. Heart rate within cows was consistent throughout the pre-stimulus (78.8 BPM) and post-stimulus (84.0 BPM) periods. In response to shock, HR was 82.4 and 82.8 baseline, 85.2 and 87.7 prep, and 82.4 and 84.7 milking for 3 and 5 ma, respectively. The number of behavioral events (lifting legs, becoming vocal, etc.) recorded during milking increased from 1.4 (pre- and post-stimulus) to 5.1 (3 ma) and 6.6 (5 ma). There was no significant change in milk yield, milking time, or time to maximum milk flow. However, there seemed to be a transient (2 days) decrease in milk yield at 5 ma. The findings of this study are anomalous in that cows appeared behaviorally to be greatly stressed by electrical shock but there was little or no effect on milking performance. Stress has been assumed to interfere with normal milk-ejection by inhibiting the release of oxytocin. The results of this study show that stress is not necessarily associated with inhibition of the milk-ejection reflex.

127.PO STRESS IN UTERO AUGMENTS BRAIN EXCITABILITY. P.B. Feuerstein* and W. Fishbein. Psychobiology Laboratory, Dept. of Psychology, The City College of the City Univ. of N.Y., New York, N.Y. 10031.

Ninety six pregnant albino mice (Carworth Farms) were subjected to either handling or severe stress during the 2nd and 3rd trimester. Stress was induced by restraint under bright lights for 90 min. daily. At birth litters were culled to 6 pups and either fostered or crossfostered. Pups were weaned at 22 days & group housed until 50 days of age. On day 50 one pup from each litter was removed and a single transcorneal electroconvulsive shock (7.0 ma, rms) was administered. Shock intensity increased on a daily basis in steps of 0.5 ma until the animal displayed a full clonic-tonic seizure.

Results reveal a significant difference between prenatally stressed offspring raised by stressed mothers & all other groups ($p<.01$). The design of the experiment reveals that stress in utero can be completely ameliorated postnatally by crossfostering to less disturbed mothers. When the effects of prenatal stress are combined with caretaking by stressed mothers, brain excitability is further increased.



128.1 THE EFFECTS OF HIGH DOSE METHYLPHENIDATE TREATMENT ON THE DEVELOPING RAT: GROWTH, DEVELOPMENT AND BEHAVIORAL MEASURES. W. J. Pizzi, E. C. Rode* and J. E. Barnhart*. Northeastern Illinois University, Chicago, IL 60625.

Stimulants are the most frequently used form of chemotherapy for the treatment of Attention Deficit Disorders (ADD, Hyperactive Child). A controversy has developed as to whether these agents, while effective in treating ADD, cause a growth deficit. Human clinical studies have been fraught with methodological difficulties. In this study we have treated rats from day 5-25 of age with varying doses (35 mg/kg x 2/day x 20 days; 100 mg/kg x 2/day x 10 days) of methylphenidate (Ritalin-R). The 35 mg/kg group showed a 6% mortality rate, while the 100 mg/kg group showed a 67% mortality rate; no deaths occurred in the control group.

Here we report on growth, developmental landmarks, and several behavioral measures. On day 5, groups did not differ in body weights; however, by day 6 the 100 mg/kg group showed significant weight losses. Measures at day 12 showed weight losses in both R groups, which continued through day 25 when necropsies were performed. R-treated rats showed decreases in femur and body lengths, as well as decreased testes, adrenal, thyroid, pituitary, and brain weights. R-treated animals showed early tooth eruption, but no differences in testes descent, air righting, or visual placing. Rats followed to 70 days of age no longer showed significant body weight differences. Animals started various activity measures between 95 and 105 days of age. No differences in activity were seen with brief activity measures (poke test and open field), but significant increases were observed in 100 mg R-treated animals during the 24-hour measure. Further analysis showed that the increased activity was due to an elevation during the 12-hour dark period. No differences were seen on the spontaneous alternation, spatial alternation, or Lashley III Maze tasks.

Differences seen at necropsy suggest that these findings may be due to the acute effects of stimulant treatment. The fact that 70 day body weights did not differ suggests that the system rebounds when the toxic agent is removed. Preliminary behavioral testing failed to show impairments in learning; however, this area deserves further exploration.

(Supported by Committee on Organized Research, Northeastern Illinois University)

128.2 REGIONAL GLUCOSE UTILIZATION IN RAT BRAIN FOLLOWING ACUTE AND CHRONIC NEUROLEPTIC ADMINISTRATION, D.K. Zucker and H.Y. Meltzer Dept. of Psychiatry, University of Chicago, Chicago, IL. 60637

Decreased glucose utilization (GU) seen in the lateral habenular nucleus (LHN) following administration of dopamine agonists, and increased GU in the LHN following single injections of dopamine antagonists (neuroleptics) suggest a dopaminergic input to the LHN (Brain Res: 194,117-124). Destruction of the entopeduncularis blocks the neuroleptic effect, suggesting that the dopaminergic input is mediated via a polysynaptic pathway involving the entopeduncularis (Soc. Neurosci. Abs. 7, 274.9). It is well known that the motoric effect of neuroleptics varies as a function of duration of administration, while the antipsychotic effects are more stable and persistent. To determine the stability of the neuroleptic effect on GU in LHN we compared animals following acute and chronic drug administration.

Three groups of rats were studied with ^{14}C-2 deoxyglucose autoradiography. Control (saline, SC, Q 2 wks), single injection (saline, SC, Q 2 wks; haloperidol, 2 mg/kg, SC, day of experiment), chronic neuroleptic (fluphenazine decanoate, 5-10 mg, SC Q 2 wks for 14 wks). GU for various structures was calculated by dividing regional optical density by mean white matter optical density. Per cent change was calculated by comparing regional GU in single injection, and chronic injection animals with controls.

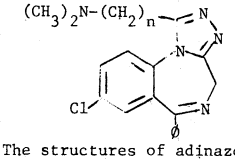
GLUCOSE UTILIZATION (% OF CONTROL)

Structure	Chronic injection	Single injection
Frontal cortex	104	92
Caudate-Putamen	123	106
N. Accumbens	111	113
Striatum-Caudate	109	100
Globus Pallus	100	93
Amygdaloid N.	91	100
Lat. Habenular N.	137	130
Med. Geniculate	109	89
Auditory Cortex	101	88
Mammillary Bodies	110	92

These findings suggest three classes of GU response to neuroleptics: an acute response only, in the medial geniculate and auditory cortex; non-tolerance in the LHN and possibly N. accumbens, and a late onset effect in the caudate-putamen. The relationship between metabolic, and other neuroleptic effects (especially antipsychotic) remains to be explored.

128.3 PHARMACOLOGICAL PROFILE AND CLINICAL ACTIVITY OF THE ANTIDEPRESSANT ADINAZOLAM, A TRIAZOLOBENZODIAZEPINE. R.A.Lahti,* J.B. Cohn,* J.P.Feighner,*W.T.Smith*and R.E.Pyke*(SPON.J.Mohrland) The Upjohn Company, Kalamazoo, MI, Irvine, CA., Encinitas, CA, Portland, OR.

The reported preclinical antidepressant and anxiolytic activity of adinazolam (Hester et al,1980) was confirmed and extended in this study. The positive clinical response with adinazolam in severely depressed patients is also discussed.



n = 1 adinazolam

n = 2 U-43,465F

The structures of adinazolam and U-43,465F are as shown. Adinazolam was effective in antagonizing metrazole-induced convulsions and in blocking stress-induced elevations in rat plasma corticosteroids. U-43,465F was weak versus metrazole and inactive in the corticosteroid test. The NE potentiating effect of U-43,465F closely paralleled the effect of the tricyclic antidepressant imipramine. Adinazolam also potentiated NE at low doses, however the degree of potentiation was less than it was for U-43,465F. Adinazolam and U-43,465F were weak at blocking the uptake of NE in the mouse heart and inactive on 5HT uptake in the mouse spleen. However, a poor correlation does exist between blockade of uptake and potentiation of NE or clinical activity (Lahti and Maickel, 1971). The potentiating effect of adinazolam on 5HTP was nonexistent, as it was for U-43,465F. The results of multiple ligand binding studies indicated that adinazolam and U-43,465F are unlike the tricyclic antidepressants in important regards. They were very effective on benzodiazepine-related binding. They did not alter binding at adrenergic, cholinergic, dopaminergic, nor serotonergic receptors as do the tricyclic antidepressants. This lack of activity is a reflection of less potential to produce very undesirable side effects.

In open-label studies of 42 depressed inpatients (HAMD ≥ 28), adinazolam was given in ascending doses up to 20-90 mg/day (tid or qid schedule). This was associated with mean improvement of 40% in depression in two days as self-rated on the Hopkins Symptom Checklist Cluster; and, in 50% of the patients, with an improvement of ≥ 50% in HAMD score in 7 days. No sign of tolerance occurred with up to 6 weeks of treatment. Sedation-related effects were modest and rare.

This class may represent a major breakthrough in safety and efficacy for the treatment of depression.

128.4 BEHAVIORAL AND BIOCHEMICAL EFFECTS OF LITHIUM ON AMPHETAMINE INDUCED DOPAMINERGIC SUPERSENSITIVITY. E. H. Rubin and G. F. Wooten. Depts. of Psychiatry and Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Chronic amphetamine can lead to a facilitation of stereotypic behavior produced by dopamine (DA) agonists. This behavioral kindling may be useful in studying mechanisms of psychosis and drug abuse as well as basic receptor functions. Lithium has been shown to block neuroleptic-induced dopaminergic supersensitivity (NIDS) and therefore we studied the behavioral and biochemical effects of chronic lithium treatment on amphetamine induced behavioral kindling. Daily injections of amphetamine (2 mg/kg SC) x 2 weeks caused a marked enhancement in the stereotypy which followed acute injections of either apomorphine or amphetamine. Chronic lithium treatment, at the same levels that block NIDS (0.71 meq/L), did not block, and in fact at certain time points, enhanced the behavioral kindling caused by daily amphetamine injections. Furthermore, chronic lithium alone led to enhanced DA agonist-induced stereotypy. For example stereotypy scores (0-6 scale) 20 min. after apomorphine (0.1 mg/kg SC) were - controls: 1.69±0.25; chronic amphetamine pretreated: 2.81±0.21; lithium pretreated: 3.16±0.25; chronic amphetamine and lithium pretreated: 4.67±0.17 (mean ± SEM, n=6-8). In order to further understand the possible mechanisms of amphetamine kindling and to see if presynaptic mechanisms influencing DA turnover are involved we examined DA, DOPAC, HVA and 3MT levels in the rat caudate and accumbens before and after single amphetamine injections in controls, amphetamine-kindled animals, lithium treated animals and lithium treated, amphetamine-kindled animals. Acute amphetamine caused an increase in DA (striatum > accumbens) and 3MT while it decreased the levels of DOPAC and HVA. The levels of DA and its metabolites and the changes which followed acute amphetamine were generally similar in controls and animals pretreated with chronic amphetamine, chronic lithium or both drugs despite the marked behavioral enhancement in stereotypy. In conclusion lithium does not block and may augment amphetamine kindling. The mechanisms of the amphetamine kindling and the lithium facilitation of stereotypic behavior apparently do not involve presynaptic mechanisms affecting levels of DA or its metabolites in the striatum or accumbens.

128.5 SIMILARITIES BETWEEN CENTRAL BINDING SITES FOR COCAINE AND IMIPRAMINE. M.E.A. Reith*, H. Sershen*, D. Allen* and A. Lajtha.Center for Neurochemistry, Rockland Research Institute, Ward's Island, New York, N.Y. 10035

Brain membranes possess binding sites that saturably bind cocaine with an affinity in the same range as brain concentrations of cocaine that are achieved by intranasal or intravenous administration. Previous work in our institute has shown that the pharmacological profile of these sites is similar to that of the neuronal serotonin uptake recognition sites, and also to that of the imipramine binding sites, which are well documented to be associated with neuronal serotonin uptake sites. These results raise the question whether cocaine binding sites are in fact located on serotonergic terminals, and whether these sites are related to imipramine binding sites.

We report here experiments in which rats were lesioned by neurotoxins aimed at serotonergic or catecholaminergic neurons. p-Chloroamphetamine and 5,7-dihydroxytryptamine decreased synaptosomal uptake of serotonin and reduced the binding of both cocaine and imipramine in the central cortex. p-Chlorophenylalanine, an inhibitor of tryptophan hydroxylase, did not affect serotonin uptake, cocaine binding, or imipramine binding. 6-Hydroxydopamine destroyed noradrenergic neurons as measured by the decrease in synaptosomal norepinephrine uptake, but left the serotonergic terminals intact, as shown by the preserved serotonin uptake; under these conditions the cortical binding of both cocaine and imipramine was unchanged. These results suggest an association of cocaine binding in the cerebral cortex with serotonergic terminals. Additional evidence in support of such an association comes from our experiments demonstrating the binding of [^{3}H] cocaine to human platelets, a model system for serotonergic neurons possessing imipramine binding sites as well. Despite their similarities, the binding sites for cocaine and the sites for imipramine are probably not identical, since the IC_{50} for imipramine in inhibiting [^{3}H] cocaine binding is much higher than that for inhibiting [^{3}H] imipramine binding. In addition, in the cerebral cortex the imipramine binding is Na^+ - sensitive, whereas the cocaine binding is not.

Supported by Health Research Council Award 11-025.

128.6 SELECTIVE BLOCKADE OF SEROTONIN UPTAKE BY 1S,4S-N-METHYL-4-(3,4-DICHLOROPHENYL)-1,2,3,4-TETRAHYDRO-1-NAPHTHYLAMINE (CP-51,974). B. Kenneth Koe, Albert Weissman*, Willard M. Welch* and Ronald G. Browne. Central Research, Pfizer Inc., Groton, CT 06340.

Marked inhibitory activity towards synaptosomal uptake of dopamine (DA), norepinephrine (NE) and serotonin (5HT) was reported previously by us for tametraline, (+)-trans (1R,4S)-N-methyl-4-phenyl-1,2,3,4-tetrahydro-1-naphthylamine (Koe, J. Pharmacol. Exp. Ther. 199:649, 1976). In the present study we found that potency for blocking monoamine uptake was notably augmented by the introduction of 3,4-dichloro substituents in the 4-phenyl ring. Unexpectedly, one of the four stereoisomers of the new compound, (+)-cis (1S,4S)-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine (CP-51,974), was found to be a remarkably potent and selective inhibitor of synaptosomal ^{3}H-5HT uptake (K_i 0.013 μM). CP-51,974 also exhibited selective 5HT uptake blockade in ex vivo and in vivo experiments. One hour after intraperitoneal administration, CP-51,974 blocked 5HT uptake ex vivo with an ED_{50} of 9 μmol/kg (3 mg/kg) and prevented the depletion of brain 5HT levels elicited by p-chloroamphetamine which must be taken up into 5HT neurons before exerting its depleting effect [ED_{50} for CP-51,974, 0.68 μmol/kg (0.24 mg/kg)]. Ex vivo uptake of ^{3}H-NE and ^{3}H-DA after CP-51,974 was much less inhibited [ED_{50} values, >100 μmol/kg (34 mg/kg) and 94 μmol/kg (32 mg/kg), respectively]. Heart uptake of ^{3}H-NE in vivo was only slightly diminished by CP-51,974 (23% decrease after 100 μmol/kg). In agreement with these neurochemical findings, CP-51,974 potentiated the behavioral effects of 5-hydroxytryptophan in mice (ED_{50}, 1-4 mg/kg p.o. for various symptoms) but did not reverse reserpine-induced hypothermia. No anticholinergic activity was seen after CP-51,974 in several in vivo tests in mice, a result consistent with its weak inhibition of ^{3}H-quinuclidinyl benzilate binding. CP-51,974 showed no stimulatory effects in rats, in contrast to the pronounced locomotor stimulation evoked by the (+)-trans (1R,4S) isomer, a potent but nonselective blocker of monoamine uptake. Therapeutic potential for CP-51,974 as an antidepressant is suggested by its actions in the following tests. CP-51,974 markedly reduced immobility ("despair") in mice in the Porsolt behavioral despair test, an effect also exhibited by many antidepressants. In addition, in rats receiving multiple doses of CP-51,974, a decrease in the response to NE was obtained in the cyclic AMP generating system of limbic forebrain slices. This down-regulation of NE receptor coupled adenylate cyclase following a multiple dosing regimen is characteristically elicited by clinically active antidepressants (Sulser, Trends Pharmacol. Sci. 1:92, 1979).

128.7 NORTRIPTYLINE TREATMENT OF POST-STROKE DEPRESSIVE DISORDER: A DOUBLE-BLIND THERAPEUTIC TRIAL. J.R. Lipsey*, G.D. Pearlson and R.G. Robinson. (SPON: P.R. McHugh) Dept. of Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, Md. 21205.

It has recently been demonstrated that 60% of left hemisphere stroke patients have clinically significant depressions (Robinson, R.G. and Szetela, B., Ann. Neurol., 9:447, 1981). Severity of depression in these patients is directly correlated with closeness of the lesion to the frontal pole. Patients with right hemisphere strokes seem unduly cheerful or have relatively minor depressions whose severity is inversely correlated with closeness of the lesion to the frontal pole (Robinson, R.G. et al., Proceedings of the 13th Princeton Conference on Cerebrovascular Disease, in press). Such findings suggest that post-stroke depressions may be symptomatic of injury to specific neural pathways. Abnormal response on the 1mg dexamethasone supression test (DST) has been shown to have a high correlation with the clinical diagnosis of endogenous depression among functionally depressed patients. Because such patients often respond to treatment with antidepressants, the present study was designed to test whether a tricyclic antidepressant can effectively treat post-stroke depression.

Patients admitted to a rehabilitation hospital following stroke are being evaluated using standardized measures of depression (Hamilton, Zung) and cognition (Mini-Mental State). Depressed patients are asked to participate in a double-blind, randomized, placebo-controlled treatment trial with nortriptyline. Consenting patients are given a 1mg DST and begin a six week treatment trial of nortriptyline or placebo (20mg/day for 1 week, 50mg/day for the next 2 weeks, 70mg/day for 1 week, and 100mg/day for the last 2 weeks). Serum nortriptyline levels are monitored weekly, and a DST is repeated at the end of treatment. Weekly standardized measures of depression and cognition are obtained during the treatment period.

At this time, 12 patients have entered our study. Preliminary results indicate 9 of these patients have initial positive DSTs. 2 of 3 placebo patients with positive DSTs remained positive at the end of treatment. 4 of 5 active medication patients had nortriptyline levels within what is considered the therapeutic range for functional depressions. Preliminary data continues to be gathered to determine efficacy of antidepressant treatment and will be presented at the meeting.

128.8 **TRICYCLIC ANTIDEPRESSANTS AND SEROTONIN MANIPULATIONS IN AN ANIMAL MODEL OF DEPRESSION UTILIZING CHRONIC INTERMITTENT STRESS.** **J.S. Soblosky and J.B. Thurmond, Neuropsychopharm. Program, Univ. of Louisville, Louisville, Ky. 40292.**

The animal model of depression utlizing chronic intermittent stress (Katz et al., 1981) has been extended to include male CD-1 male mice. The drugs tested were 3 tricyclic antidepressants (TCA) amitryptyline (AMI), desimipramine (DMI) and chlorimipramine (CHLOR) in addition to fluoxetine, a specific serotonin (5-HT) uptake inhibitor. All were administered acutely or chronically (14 days) in 5 mg./kg. doses. After chronic administration groups were also challenged with either 5-HTP (50 mg./kg.) or PCPA (200 mg./kg. x 4 days). Motor activity was was assessed via automatically recording motimeters and exploration via a modified holeboard. The biogenic amines NE, DA and 5-HT as well as the metabolites DOPAC, HVA and 5-HIAA were assessed via HPLC with EC detection and coticosterone (CS) via fluorometry.

Acutely, all of the drugs caused decrements in all behavioral measures in both stress and non stress conditions. When given chronically, AMI, DMI and CHLOR resulted in improvement in all behavioral measures, while fluoxetine was ineffective. PCPA was found to increase activity in response to stress but to have negative effects on exploration in nonstress conditions and no effects in stress conditions. 5-HTP was found to be ineffective in activity measures but to increase exploration in stress conditions. The PCPA effects on motor activity were unaffected by the 3 TCAs but decreased by fluoxetine. The PCPA effects on exploration were ameliorated by all the drugs. The 5-HTP effects on exploration were not affected by the 3 TCAs but were enhanced by fluoxetine.

Acute stress was shown to increase 5-HT and 5-HIAA and decrease NE. Chronically stressed mice were shown to still exhibit an increase in 5-HIAA but an increase in NE. Acutely, the TCAs and fluoxetine either decreased or had no effect on 5-HIAA. Chronically, the TCAs increased but fluoxetine decreased 5-HIAA.

The CS responses were normalized by all the drugs. Generally 5-HTP was stimulatory but PCPA inhibitory in the response. When combined with the 3 TCAs, PCPA effects were unaffected but prevented by fluoxetine. The 5-HTP effects were prevented by AMI and potentiated by fluoxetine.

The model demonstrated that AMI, DMI and CHLOR are effective and fluoxetine is ineffective in reversing behavioral changes caused by chronic intermittent stress, suggesting that 5-HT uptake inhibition is not necessary. 5-HT involvement is suggested by the effects of PCPA and 5-HTP in addition to the normalizing of their effects by the TCAs. Receptor alterations are a probable alternative.

128.9 RAT INTERNAL CAPSULE LESION: FURTHER CHARACTERIZATION OF ANTIDEPRESSANT SCREENING POTENTIAL. M. R. Szewczak*, S. Fielding and M. Cornfeldt*. Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

As previously reported (Cornfeldt et al., Fed. Proc., 41: 1066, 1982), a lesion of the internal capsule in the region of the diencephalic-telencephalic border causes a decrement in bar pressing for reinforcing brain stimulation from electrodes placed in the medial forebrain bundle. Clinical antidepressant agents have been shown to reverse this decrement over a period of several days contrasted with a relative lack of effect in non-lesioned control rats. This system can be described functionally as a hypoactive reward system. All drugs were administered i.p. once daily for five or nine days, 30 min. prior to self-stimulation testing. Desipramine (10 mg/kg) increased responding 19% over nine days, nisoxetine (10 mg/kg) increased responding 52% over five days, pyrazidol (5 and 10 mg/kg) +28% and +11% over five days, respectively, iproniazid (10 mg/kg) +17% over five days and morphine sulfate (2.5 mg/kg) +29% over five days. Yohimbine (2.5 mg/kg) and propranolol (5 mg/kg) were without effect. These data provide further evidence of the value of this model of depression for screening clinical antidepressant candidates.

128.10 BEHAVIORAL AND BIOCHEMICAL EFFECTS OF CHRONIC INFUSION OF THE NOVEL ANTIDEPRESSANT, BUPROPION, IN RATS. Barrett R. Cooper and Robert F. Butz*. Departments of Pharmacology and Medicinal Biochemistry, Burroughs Wellcome Co., Res. Tri. Pk., N.C. 27709.

Behavioral changes and alterations in the synthesis and metabolism of monoamines after acute injection or chronic infusion of bupropion in rats were compared. Serum levels of bupropion after i.p. injection or at the termination of infusion periods were used as a basis for drawing comparisons. One hour after i.p. bupropion administration, a dose-related reduction of time spent immobile in the Porsolt antidepressant test was observed after doses of 3 to 12 mg/kg, while a significant elevation of locomotor activity occurred after a 25 mg/kg dose. Biochemically, this dose range had no effect on monoamine levels but significantly elevated ^{3}H-norepinephrine synthesis from ^{3}H-tyrosine and significantly decreased ^{3}H-dopamine synthesis from ^{3}H-tyrosine. DOPAC levels in brain were also reduced. Acute bupropion (25 mg/kg) blocked dopamine reuptake in vivo and also elevated 5HT turnover and 5HIAA. Chronic (6 day) infusions of bupropion (2.5, 5 and 10 mg/kg/hr) delivered by subcutaneously-implanted, osmotic pumps achieved bupropion serum levels on day 6 similar to those attained 1 hr after single i.p. injections of 3, 6 and 25 mg/kg. In these rats, no significant changes in norepinephrine or 5HT turnover or metabolism were observed suggesting adaptation to the effects of bupropion on these neural systems. A significant dose-related decrease in dopamine synthesis and metabolism was observed in infused rats. Behaviorally, the locomotor stimulant effects of bupropion associated with higher serum levels (e.g., equivalent to the 25 mg/kg i.p. dose) largely disappeared after 48 hrs. of infusion and there were no differences between infused controls and rats infused with bupropion when locomotor activity was measured on day 6. However, infused animals still responded in the Porsolt test for antidepressant activity. It is concluded that acute treatment with bupropion alters turnover of NE, 5HT and DA and that high doses are associated with locomotor stimulation. During chronic treatment, adaptation occurs to the actions of bupropion on measures of NE and 5HT turnover and on locomotor activity but not on measures of the turnover and metabolism of DA or on measures of antidepressant activity.

128.11 SPECIFIC BINDING OF ^{3}H-IMIPRAMINE TO C_6 ASTROGLIOMA CELLS. P.M. Whitaker and J.J. Warsh. Section of Biochemical Psychiatry, Clarke Institute of Psychiatry, Toronto, Ontario, CANADA.

Tricyclic antidepressants are known to inhibit the uptake of neurotransmitters into brain cells. Radiolabelled forms of these drugs, such as ^{3}H-imipramine, are thought to be useful in labelling the neurotransmitter uptake site or a site closely associated with it.

Brain astroglial cells have the capacity to take up serotonin, however binding studies have not yet been successful in labelling these transporting sites with tricyclic antidepressants. We now report the presence of high affinity ^{3}H-imipramine binding sites on the mouse astroglioma cell line, C_6.

C_6 cells were grown in Falcon flasks using F_{10} liquid media supplemented with 2.5% fetal calf serum and 15% horse serum. Seven day old cultures were used in the binding assays. The cells were rinsed twice with buffer (50 mM. TRIS HCl buffer, containing 120mM. NaCl and 5mM. KCl, final pH 7.4) before being scraped from the flasks with a rubber policeman. The cell suspension was then washed twice with buffer and centrifuged at 50,000 g. for 20 min. The final suspension was adjusted to an approximate protein concentration of 1.0 mg./ml. The suspension was used immediately in the binding assay.

For determination of ^{3}H-imipramine binding the following aliquots were incubated in glass test tubes: 0.10 ml. radioligand (final concentration 0.15 to 1.5 nM.); 0.20 ml. of cell homogenate; and 0.10 ml. of buffer or buffer containing unlabelled desipramine to define specific binding (final concentration 10^{-4} M.). After a two hour incubation on ice, a 0.20 ml. aliquot of each test tube was diluted into 4.0 ml. of buffer and rapidly filtered under vacuum through Whatman GF/B filters. The filters were washed three times with 4.0 ml of buffer each time.

Scatchard analysis indicated a single class of binding sites with an appropriate K_D of 1.7 nM. The maximum number of sites was estimated to be 202 fmoles/mg. protein. Specific binding of ^{3}H-imipramine was competitively inhibited by other tricyclic antidepressants, several serotonin uptake inhibitors and antihistamines.

This work demonstrates that ^{3}H-imipramine labels a site on astroglial cells, a finding which may be significant in understanding antidepressant drug action.

(Supported by The Medical Research Council of Canada and The Ontario Mental Health Foundation).

128.12 SELECTIVE 5-HT_2 RECEPTOR BLOCKADE: PHARMACOLOGIC STUDIES OF MJ 13754, A NONTRICYCLIC ANTIDEPRESSANT CANDIDATE. Duncan P. Taylor, Michael S.Eison, L. A. Riblet, D. L. Temple Jr., J. P. Yevich*, and David W. Smith*. Preclinical CNS Research, Pharmaceutical Research and Development Division, Bristol-Myers Company, Evansville, Indiana 47721.

The potential antidepressant activity of MJ 13754, known chemically as 2-(3-[4-(3-chlorophenyl)-1-piperazinyl]propyl)-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-3H-1,2,4-triazol-3-one, was first identified by its ability to reverse reserpine-induced ptosis in mice (ED_{50} = 19 mg/kg, p.o.). MJ 13754 was a potent inhibitor (IC_{50} = 20 nM) of [^{3}H]spiperone bound *in vitro* to sites on membranes prepared from rat frontal cortex. This suggested a serotonergic mechanism (5-HT_2 or S_2) of action. Further support for this suggestion came from the ability of MJ 13754 to increase the duration of immobility in the forced swimming model of behavioral despair. Similar activity in this test has been reported for other serotonergic agents. Potential antidepressant activity was also evidenced by the ability of MJ 13754 to decrease the number of binding sites for [^{3}H]spiperone in frontal cortical preparations after chronic administration (100 mg/kg for 11 days, i.p., or 28 days, p.o.). Repeated MJ 13754 treatment produced no change in *in vitro* binding for α_2-adrenergic, histaminergic, or dopaminergic ligands. Unlike the conventional tricyclic antidepressants, MJ 13754 lacked anticholinergic activity *in vivo* (did not prevent physostigmine-induced lethality) and *in vitro* (inhibition of ^{3}H-QNB binding). More importantly, MJ 13754 had low affinity for α_1-adrenergic and histaminic receptor binding sites *in vitro*, predicting less sedation clinically. Consonant with these data were MJ 13754's low potency in the prevention of histamine- or norepinephrine-induced lethality, in the potentiation of the ethanol- or hexobarbital-induced loss of the righting reflex in rats and mice, in the reduction of spontaneous motor activity, and in the reduction of rotorod performance. MJ 13754 did not inhibit monoamine oxidase activity *in vivo* or *in vitro*. MJ 13754 lacked activity at benzodiazepine and γ-aminobutyric acid binding sites *in vitro* and did not interact with α_2-adrenergic or dopaminergic receptors *in vivo* or *in vitro*. These studies suggest that MJ 13754 should be a clinically effective antidepressant without anticholinergic, antihistaminic, or sedative side effects.

128.13 BRAIN REGIONAL DISTRIBUTION AND PLASMA LEVELS OF TRICYCLIC ANTIDEPRESSANTS IN THE RAT. L. L.Hsu and S. Tang* Department of Psychiatry and Behavioral Sciences, University of Texas Medical Branch, Galveston, Texas 77550

To determine whether the plasma levels of tricyclic antidepressants (TA's) actually represent their concentrations at the site of action in the brain, we have examined the possible correlations between plasma levels of TA's and their distribution in brain regions. A sensitive and reliable assay method has been developed for measuring TA levels in brain regions and in plasma using gas liquid chromatography with nitrogen-specific detection.

Adult female Fischer 344 rats (200 g body weight) were used in this study. Rats were administered with amitriptyline (AMI) intraperitoneally at various doses (5, 7.5 and 10 mg/kg) and sacrificed by decapitation half an hour later. Blood from the whole body was collected in heparinized beakers and centrifuged at 2 K rpm for 20 min. to separate plasma. Brain regional tissues were dissected over ice and homogenized in appropriate amounts of 0.1 N HCl. The homogenates were centrifuged at 20 K rpm for 15 min. Aliquots of the supernatants and plasma were alkalinized with 5N NaOH and the free AMI and nortriptyling (NT) were extracted into heptane/isopropyl alcohol (H/I, 9:1) according to the procedure of Bertrand et al (Clin. Biochem II (3):117, 1978) with modifications. The drugs in the organic layer were subsequently back extracted into small volumes of 0.1 N HCl. After the aqueous layer was sufficiently washed with organic solvents under acidic condition, it was alkalinized with 4N NaOH and the drugs were extracted into 0.8 ml of H/I. An aliquot (0.6 ml) of the H/I layer was evaporated under air till driness. Twenty-five µL of H/I was added to redissolve the residue and 2 µL of the solution was used for quantitative analysis of AMI and NT by gas chromatography. A varian 3700 GC with a nitrogen-specific detector was used. Operating conditions were: a 3 feet long, 2 mm I.D. glass coiled column containing 3% OV-17 on chromosorb W 100/200 maintained at 230°C; injector temperature at 220°C and detector temperature at 270°C with the beads current adjusted to maximum sensitivity.

Our results indicate that, at half an hour following a single dose (10 mg/kg) of AMI, the drug was already taken up by all 11 brain regions examined. However, no metabolite (NT) of AMI was detected in the brain or plasma at this time. The highest concentration of AMI was observed in septum (26 U, U = ng/mg protein) followed in descending order by hypothalamus (22 U), choroid plexus (18 U), olfactory bulb (9U), pituitary (6.4 U), cerebral cortex (6.3 U), hippocampus (5.7 U), corpus striatum (5.4 U), cerebellum (5 U) and brainstem (4.4 U). Midbrain-diencephalon had the lowest level (4.2 U). The plasma level of AMI was 58 ng/ml. Levels of AMI in brain regions and plasma decreased with decreasing doses.

128.14 10-HYDROXYNORTRIPTYLINE PLASMA CONCENTRATIONS IN ELDERLY DEPRESSIVES. R. C. Young, G. S. Alexopoulos*, M. W. Manley*, A. Dhar*, H. Kutt* (SPON: C. Shamoian). Depts. of Psychiatry and Neurology. Cornell Univ. Med. College, New York, N. Y. 10021.

During chronic therapy with nortriptyline (NT), plasma NT levels vary widely between individuals receiving equivalent doses. This variation accounts for some of the observed inter-individual difference in antidepressant response and toxicity. Hepatic hydroxylation is a major metabolic pathway for NT. 10-hydroxynortriptyline (10-OH-NT) is biologically active and may contribute to therapeutic and toxic effects. In the elderly, the ratio of plasma 10-OH-NT/NT may be altered by factors including decreased renal excretion of 10-OH-NT.

Elderly (>age 60) inpatients with major depression were treated with NT in steady state doses of 50-150 mg/day. Clinical measures and plasma samples were obtained weekly. Plasma concentrations of NT and of the unconjugated (E) and (Z) isomers of 10-OH-NT were determined simultaneously by high pressure liquid chromatography with ultraviolet absorption detection. Comparisons were made with values obtained in young adult (<age 40) inpatients with major depression receiving NT in equivalent doses.

In a preliminary analysis, elderly depressives (n=9) had a mean plasma NT concentration of 125 ng/ml ± 44 ng/ml (S.D.) and a mean unconjugated (E)10-OH-NT concentration of 247 ng/ml ± 117 ng/ml. The mean ratio of (E)10-OH-NT/NT was 2.28 ± 1.23 and ranged from 0.19 to 4.03. The mean unconjugated (Z)10-OH-NT concentration was 45 ng/ml ± 3 ng/ml. The mean ratio of (Z)10-OH-NT/NT was .43 ± .21. The coefficients of variation of (E)10-OH-NT/NT in individual patients over four serial samples averaged 14%.

In young adult depressives (n=5) the mean plasma NT concentration was 117 ng/ml ± 24 ng/ml. (E)10-OH-NT concentrations averaged 133 ng/ml ± 77 ng/ml. The mean ratio of 10-OH-NT/NT was 1.17 ± 0.62 and ranged from 0.30 to 1.96. The coefficients of variation were the same as in elderly patients.

In elderly depressives the ratio of (E)10-OH-NT/NT varies widely between individuals but shows little intraindividual variation. This ratio tends to be higher in elderly than in young adult depressives. The contribution of (E)10-OH-NT to therapeutic and toxic effects in the elderly is being investigated.

(Supported by award from Dept. of Psychiatry, CUMC.)

128.15 EFFECTS OF CHRONIC DIETARY LITHIUM EXPOSURE ON RAT BRAIN ENKEPHALIN SYSTEMS. David A.Staunton, Scott N.Deyo, William J.Shoemaker, Aaron Ettenberg and Floyd E.Bloom, The A.V.Davis Ctr.for Behav.Neurobiol., The Salk Inst., La Jolla, CA 92037.

An earlier report (PNAS, 75:2991, 1978) indicated that daily Li treatment (i.p.) induced a transitory rise in the met-enkephalin immunoreactive (IR) content of rat globus pallidus. Recently, we have undertaken a more extensive examination of the effects of Li on rat brain enkephalin systems using two Li-containing dietary formulations developed in this laboratory; a lower strength diet containing 30 mmol Li/ kg (brain Li = 0.4-0.55 mEq/L) and a high strength diet with 40 mmol Li/kg (brain Li = 0.7-1.0 mEq/L). We sought to relate alterations of leu-enkephalin I.R. (l-enk-IR) content to changes of in vitro K^+-stimulated, Ca^{+2} dependent release of l-enk-IR. After 7 days on the lower strength diet there were no significant alterations of l-enk-IR content in any of the brain regions examined, nor was there an effect on the release of l-enk-IR from globus pallidus slices. After 14 or 21 days of ad lib feeding with the lower strength diet, l-enk-IR levels in the globus pallidus and nucleus accumbens were significantly elevated by 30%. However, 3 weeks of Li treatment with the high strength diet did not lead to alterations of l-enk-IR content in any of the brain regions examined. In contrast, the release of l-enk-IR was potentiated (by about 40%) only in the subjects fed the lower strength diet for 14 days or the high strength diet for 21 days, at which times the brain Li levels were relatively high compared to the other treatment groups. We conclude that the potentiating effect of long-term Li on l-enk-IR release is not necessarily associated with increased content of the peptide which may adapt to the presence of Li^+, thus giving rise to the transiency. However, increases in l-enk-IR release appears to require minimum Li^+ brain levels of 0.5 mEq/L. In further studies, we observed that hot-plate (55°C) escape latency was significantly increased in the animals fed the high strength Li diet for 21 days, an effect that was partially reversed by naltrexone (1 mg/kg, s.c.) pretreatment. Moreover, rats from this treatment group showed a greater morphine-induced (2.5 mg/kg, i.p.) elevation of escape latency than controls. However, naltrexone less effectively blocked the effect of morphine in the Li-fed subjects than in the controls. Yet there was no difference in D-ala-D-leu-enk binding affinity and sites. In conclusion, prolonged lithium administration leads to transiently increased l-enk content and, when brain Li > 0.5 mEq/L, to a potentiation of endogenous enkephalin release. Li could be directly acting on presynaptic enkephalin-containing nerve terminals, or indirectly via other neurotransmitter systems. Supported by MH 08080 and NIDA 01785.

128.16 EFFECTS OF REPEATED ZIMELIDINE ADMINISTRATION ON SEROTONINERGIC NEUROTRANSMISSION: SINGLE-CELL STUDIES IN THE RAT. P. Blier* and C. de Montigny (SPON: L. Descarries) Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec, Canada H3C 3T8.

Zimelidine (Z), a selective serotoninergic (5-HT) reuptake blocker, is a clinically effective antidepressant. However, its rapid action on reuptake is in apparent discrepancy with its delayed clinical efficacy and data on the effect of its chronic administration on 5-HT neurotransmission has never been provided. To investigate these aspects, male Sprague-Dawley rats (175-275 g) received daily injection of Z (5 mg/kg, i.p.) for 2, 7 or 14 days and electrophysiological experiments were carried out 24 h after the last injection under chloral hydrate anesthesia (400 mg/kg, i.p.).

In a first series of experiments, unitary recordings of 5-HT neurons were obtained from the mesencephalic dorsal raphe nucleus. After 2 days of Z treatment, the number of 5-HT units discharging spontaneously was greatly reduced. After 7 days of treatment, the number of active 5-HT neurons had returned to normal values but their firing rate was slower than in control animals. After a treatment of 14 days, both the number of active 5-HT units and their mean firing rate were within normal range. At that time, the responsiveness of 5-HT neurons to i.v. LSD was assessed: the ED_{50} of LSD was 2-3 fold greater than in control rats indicating that their autoreceptors had desensitized.

In a second series of experiments, five-barelled micropipettes were used to record CA_3 hippocampal pyramidal neurons in controls and in rats treated for 14 days with Z. The response of these neurons to the electrical stimulation (80 µA, 0.5 ms, 0.8 Hz) of the ventro-medial 5-HT pathway was assessed from peristimulus time histograms. The responsiveness of the same cells to iontophoretic applications of 5-HT creatinine sulfate (2 or 0.5 mM in 0.2 M NaCl, pH: 4) and GABA (50 mM in 50 mM NaCl, pH: 4) were estimated using the IT_{50} method (current time required to obtain a 50% decrease of firing rate). The IT_{50} values for 5-HT and GABA were not modified by the Z treatment, confirming earlier results. The suppression of firing of pyramidal neurons induced by stimulation of the ascending 5-HT pathway was significantly greater in Z treated rats than in controls.

It is concluded that long-term Z treatment enhances the efficacy 5-HT neurotransmission in the hippocampus. However, the reuptake blockade by Z cannot result in an enhanced 5-HT neurotransmission until 5-HT neurons resume a normal electrical activity. This sequence of events may well account for the delayed antidepressant effect of zimelidine in major depression.

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128.17 CHRONIC RESTRAINT STRESS ELICITS A POSITIVE ANTIDEPRESSANT RESPONSE ON THE FORCED SWIM TEST. J. E. Platt and E. A. Stone. Dept. of Psychiatry, New York Univ. Med. Ctr., New York, NY 10016.

Certain effects of chronically administered antidepressant agents in animals have been shown to be similar to those produced by chronic stress. These include reductions in the density of brain beta adrenergic receptors, decreases in norepinephrine-sensitive adenylate cyclase activity and the prevention of learned helplessness. Based on these similarities, we have proposed the hypothesis that antidepressant therapy is a form of adaptation to stress that works by mimicking the desensitizing action of stress at brain beta adrenergic receptors, thereby causing increased resistance to emotional stress (Stone, E. A., Res. Comm. Psychol. Psychiat. Behav., 4:241, 1979). Porsolt and his colleagues (Porsolt, R. D. et al., European J. Pharmacol., 47:379, 1978) have developed a behavioral test for antidepressants that utilizes the observation that rats forced to swim in an escape-proof cylinder eventually assume a characteristic immobile posture, the onset of which is delayed by pretreatment with antidepressants. We used this procedure to test the foregoing hypothesis and predicted that adaptation to chronic stress would result in a positive antidepressant response on the forced swim test.

Separate groups of male Sprague-Dawley rats (9 per group) were subjected either to 11 days of immobilization stress for 2.5 hrs. per day using the procedure of Kvetnansky and Mikulaj (Endocrinol., 87:738, 1970), to one 2.5 hr. period of immobilization on the test day, or to chronic antidepressant treatment consisting of 2 daily i.p. injections of desmethylimipramine HCL (DMI) (10mg/kg) for 10 days followed by 1 on the 11th day. Control groups received either 11 days of handling, vehicle (saline) injections twice daily or no handling. Testing was done using the procedure of Porsolt. Briefly, on the day preceding the test, rats were swum individually for 15 min. in 15cm of water (25°C) and were then allowed to dry. Twenty-four hours later they were swum again for a 5 min. test period while immobility was recorded. Testing occurred 1 hr. after the final injection or stress.

The duration of immobility in the chronic restraint group (53.2 ± 11.8 sec.) and in the chronic DMI group (44.9 ± 7.2 sec.) was significantly less than in nonhandled controls (120.9 ± 17.6 sec.), $p < .05$ by the Dunnet test after a 1-way ANOVA ($F(5,48) = 9.10$, $p < 0.0001$). No effect on immobility was found in the handled (151.2 ± 15.5 sec.), vehicle injected (113.8 ± 12.6 sec.) or acutely restrained rats (101.4 ± 15.0 sec.). These results support the hypothesis that adaptation to stress and antidepressant therapy are similar processes and are likely to be mediated by similar central neurochemical processes.

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128.18 THE BEHAVIORAL PHARMACOLOGY OF AY-27,110, A CHEMICALLY NOVEL DOPAMINERGIC AGONIST. K. Voith* (SPON: G. Metcalf). Dept. of Pharmacol., Ayerst Research Laboratories, Montreal, Canada.

AY-27,110 (S(-) 2-[4-[2-hydroxy-2-(3',4'-dimethoxyphenyl) ethyl]-1-piperazinyl]-2,4,6-cycloheptatriene-1-one, HCl) is the pharmacologically active enantiomer of a novel, troponylpiperazine derivative. During initial testing, AY-27,110 exhibited pharmacological activity characteristic of dopaminergic agonists and thus was evaluated subsequently as a potential antiparkinson agent.

In animal models that mimic Parkinson's disease with regard to degenerative changes, dopamine deficiency and supersensitive postsynaptic receptors, the compound was active at low doses. Thus, AY-27,110 (1.25-10 mg/kg p.o.) induced contralateral rotations in rats with a unilateral 6-OHDA-induced lesion of the nigro-striatal pathway. In rats, rendered akinetic by the bilateral injection of 6-OHDA into the anterolateral hypothalamus, AY-27,110 (2.5-10 mg/kg s.c.) brought about a well-coordinated locomotor activity.

In normal animals, AY-27,110 (5-20 mg/kg p.o.) induced dose-dependent stereotyped behavior. However, only the weaker components of stereotypy were seen, namely continuous sniffing and repetitive head movements, while intense gnawing, licking and biting were absent. In separate experiments, doses as low as 0.2 mg/kg s.c. significantly inhibited locomotor activity while higher doses caused stimulation, suggesting that AY-27,110 was capable of interacting with both dopamine autoreceptors and postsynaptic receptors, respectively.

The aforementioned effects of AY-27,110 were antagonized by haloperidol and pimozide, indicating the dopaminergic nature of the responses. However, pretreatment with either α-methyl-p-tyrosine or reserpine did not alter the effects of AY-27,110, indicating that they are independent of presynaptically available dopamine.

In all of these experiments AY-27,110 was compared to bromocriptine and was found to be equipotent or more potent on a mg/kg basis.

The results will be discussed (a) with regard to the potential therapeutic advantages of AY-27,110 in the treatment of Parkinson's disease and (b) in relation to present-day concepts of the mechanism of dopaminergic agonist activity.

128.19 CESIUM ION AUGMENTS CHLORPROMAZINE ATTENUATION OF CONDITIONED AVOIDANCE RESPONDING WITHOUT OVERT INCREASE IN CENTRAL TOXICITY. C. Pinsky* and R. Bose*. (SPON: J.R. Wilson). Department of Pharmacology and Therapeutics, University of Manitoba, Faculty of Medicine, 770 Bannatyne Ave., Winnipeg, Manitoba, Canada R3E 0W3.

We have previously shown cesium ion to have a CNS activity profile with some similarities to that of phenothiazine antipsychotic agents (Bose & Pinsky, The Pharmacologist, 1980; Proc. Can. Fed. Biol. Soc., 1981). For the present study groups of male Swiss-Webster albino mice were pretrained on the pole-climbing conditioned avoidance response (CAR) paradigm. Two groups of these mice were treated with CsCl at 1.0 mEq Cs^+ kg^{-1} da^{-1} i.p. x 3 da and at 5.0 mEq Cs^+ kg^{-1} i.p. x 1 injection; a third group (controls) received concomitant equivolume equifrequent injections of normal saline. All mice received an injection of chlorpromazine.HCl (CPZ) (0.1 or 0.5 mg kg^{-1} i.p.) at 1 hr prior to testing CAR on day of comparison trials. Avoidance performance after CPZ injection was compared between the different cesium- and saline-treated groups. All treatments with cesium significantly enhanced the ability of CPZ at both doses to impair CAR performance. There was no concomitant increase in extrapyramidal effects of either substance, as evidenced by the lack of any notable bradykinesia, limb rigidity, immobility or catatonia appearing with the cesium augmentation of CPZ-induced CAR attenuation. In parallel experiments the abdominal aorta was cannulated in anesthetized mice (urethane 1.0 g kg^{-1} i.p.) and CsCl solution (1.0 to 5.0 mEq Cs^+ kg^{-1}) was instilled into the peritoneal cavity. This produced a rapid rise in mean blood pressure. Repeated instillations resulted in tachyphylaxis to the pressor effect of CsCl. In other mice acute adrenalectomy or mecamylamine pretreatment blocked the pressor effect. This suggests a cesium-induced adrenal medullary release of catecholamines, similar to that seen with reserpine. It might be possible also that cesium produces its neurolepticlike effect via a depletion-induced decrease in central catecholaminergic tone, as does reserpine.

We interpret our results as indicating that cesium and CPZ interact indirectly on CAR by altering two separate yet functionally related central sites. This selectivity of effective action as compared with toxic action may make cesium ion of interest as an adjunct in antipsychotic therapy with CPZ or similar antipsychotic agents. This work was supported by NSERC and MRC Canada, by NTEP program of Employment and Immigration Canada and by a Fellowship award to R.B. from the Manitoba Health Research Council.

128.20 **AHR-8559, A POTENTIAL ANTIEPILEPTIC AGENT.** David N. Johnson and Ewart A. Swinyard*, A. H. Robins Research Labs, Richmond, VA 23261-6609 and U. Utah, Salt Lake City, UT 84112.

AHR-8559 (N-methyl-3-[3-(trifluoromethyl) phenoxy]-1-azetidinecarboxamide) was effective in mice and rats in preventing convulsions induced by electrical (maximal electroshock) and chemical (sc Metrazol, bicuculline, picrotoxin) stimuli. The profile of activity of AHR-8559 most closely resembled those of phenobarbital and valproic acid, and differed from those of phenytoin and ethosuximide. The potency of AHR-8559, administered IP, was approximately 1/2 to 1/5 that of phenobarbital; compared with valproic acid AHR-8559 was approximately 2 to 6 times more potent. The drug was well absorbed orally; the oral ED_{50}s were approximately 2 to 3 times greater than the intraperitoneal ED_{50}s. Tolerance to the anticonvulsant effect did not develop in rats when the drug was administered daily for 5 days. Behavioral studies in mice suggested that the drug produced CNS depression (decreased spontaneous motor activity, hyporeflexia, etc.) in doses of 30-300 mg/kg, IP. Spontaneous cerebral cortical activity in curarized cats was slowed by AHR-8559 at 40 mg/kg, PO, although sleep spindles were not seen. In freely-moving cats with chronically implanted cerebral cortical electrodes, neither behavior nor the EEG was altered when the drug was given at 20 mg/kg/day for 5 days. AHR-8559 blocked the polysynaptic linguomandibular reflex in chloralose-anesthetized cats without altering the monosynaptic patellar reflex. The drug did not increase milk consumption in naive rats, nor did it attenuate suppressed responding in the Geller and Seifter model of antianxiety drug testing. These data suggest that AHR-8559 is an anticonvulsant agent with central muscle relaxant activity but without anxiolytic properties. (Supported, in part, by a [Contract No1-NS-1-2347] from the Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke [NINCDS], NIH, to Dr. Swinyard).

128.21 IS THE BZ_1 SELECTIVE AGENT CL 218,872 A NON-SEDATIVE ANXIOLYTIC? D.W. Straughan, N.R. Oakley* and B.J. Jones*. Pharmacology Department, Glaxo Group Research Ltd., Greenford, Middlesex UB6 OHE, U.K.

The triazolopyridazine CL 218,872 has been claimed to have a non-sedative but anxiolytic profile in animal studies and to bind preferentially to a sub-population of benzodiazepine (BZ) receptors (Lippa et al., Pharmac. Biochem. Behav., 10; 831-843, 1979). This selectivity can be demonstrated in vitro as a greater inhibition of ^{3}H-flunitrazepam binding by CL 218,872 in the BZ_1-rich cerebellum than in the cerebral cortex (mixed BZ_1 and BZ_2). We have now observed the same phenomenon of differential binding in vivo when ^{3}H-flunitrazepam is injected intravenously in mice dosed previously with CL 218,872, although it is evident only at high doses. Thus, 128mg/kg p.o. inhibited ^{3}H-flunitrazepam binding in cerebellum by 81% and in cerebral cortex by 58%, whereas 8mg/kg p.o. produced 21% inhibition in cerebellum and 18% in cerebral cortex.

We have been unable to confirm that CL 218,872 has a non-sedative but anxiolytic profile in the rat. In male Sprague-Dawley (Charles River) rats, CL 218,872 had an MED of 10mg/kg p.o. in the water-lick conflict test and significantly reduced locomotor activity in rats at 20mg/kg p.o. Thus, CL 218,872 had a similar profile to diazepam although it was only one-fifth as potent. In Glaxo CRH male mice, CL 218,872 had an ED_{50} of 58mg/kg p.o. against footshock induced fighting, an MED of 50mg/kg p.o. in the wire manoeuvre test and it significantly reduced locomotor activity at 25mg/kg p.o. In similar tests diazepam had an ED_{50} of 1.2mg/kg, an MED of 1.25mg/kg and produced a significant reduction of locomotor activity at 10mg/kg. Diazepam was also 20 times more potent than CL 218,872 at potentiating pentobarbitone-induced narcosis in mice.

Our failure to find convincing evidence of major qualitative behavioural differences between CL 218,872 and diazepam in rats and mice could reflect the small difference in affinity shown by CL 218,872 for the two principal BZ receptor sub-types or the direction of selectivity. However, it is possible that differences in the strains of animals used and in the protocol influence the sensitivity of the tests and the capacity to detect differences in behavioural profiles.

128.22 INTRACRANIAL SELF-STIMULATION RESPONSE DECREMENT PATTERNS DIFFERENTIATE DRUG-INDUCED PERFORMANCE DEFICITS FROM EFFECTS ON REWARD. H.M. Fenton*, N.R. Hall* and J.M. Liebman (SPON: M. Roffman). Res. & Dev. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

It has been suggested that neuroleptics cause an "extinction"-like decrement in intracranial self-stimulation (ICSS) response rates (Fouriezos et al., J. Comp. Physiol. Psychol. 92:661, 1978). According to these authors, rats treated with pimozide or d-butaclamol bar-pressed at normal rates at the start of an ICSS session but then virtually ceased responding after several minutes. It was inferred that the effects of neuroleptics on ICSS were analogous to withdrawal of reward. In contrast, they showed that phenoxybenzamine, an alpha-adrenoceptor antagonist devoid of neuroleptic properties, reduced responding uniformly as the test session progressed. This pattern was interpreted to indicate simple performance deficit. We have examined the response decrement patterns (RDPs) produced by a wider variety of psychotropic drugs, and have confirmed the value of this analysis for interpreting drug effects on ICSS.

Rats were trained to bar-press for lateral hypothalamic ICSS on a continuous reinforcement schedule. Current intensities were adjusted to yield near-maximal baseline response rates (50-120 responses per min). When responding stabilized, drug testing began. Response rates during the first 4 min and the final 4 min of the 15 min session were statistically compared.

Haloperidol (0.1-0.3 mg/kg) and metoclopramide (3-10 mg/kg), two structurally different dopamine antagonists, each produced an "extinction"-like RDP. At representative doses, response rates during the last 4 min were less than 10% of those during the first 4 min. In contrast, methocarbamol (125-200 mg/kg), a skeletal muscle relaxant, and prazosin (1-5.4 mg/kg), an alpha-1 adrenoceptor antagonist, both reduced responding uniformly throughout the test session. Such an RDP, therefore, appears to reflect a performance deficit, as previously suggested.

Interestingly, the RDP produced by clozapine (3-10 mg/kg), an atypical antipsychotic, resembled the RDPs produced by prazosin and methocarbamol rather than those resulting from neuroleptic treatment. Thus, clozapine's suppression of ICSS may not depend on its dopamine antagonist properties. Baclofen (3-10 mg/kg) and clonidine (0.03-0.3 mg/kg) each produced a moderate decrement of responding in the final 4 min (to 35% of the initial rate), in agreement with previous reports that these agents may possess some selectivity for reward aspects of ICSS. It is concluded that analysis of ICSS RDPs can differentiate various classes of psychotropic drugs that attenuate bar-pressing for ICSS.

128.23 EFFECT OF ACUTE CLONIDINE ADMINISTRATION ON STRIATAL DOPAMINE AUTORECEPTORS. B.S. Glaeser, J.C. Berry*, W.C. Boyar*, and R.A. Lovell. Research and Development Department, CIBA-GEIGY Corporation, Summit, N.J. 07901.

In vivo striatal dopamine autoreceptor activity was assessed after the acute administration of the α_2-adrenoreceptor agonist clonidine. Dopamine autoreceptor activity was measured by the inhibition of gamma-butyrolactone (GBL)-induced accumulation of the dopamine precursor, dopa (Walters and Roth, Naunyn Schmiedeberg's Arch. Pharmacol. 296: 5, 1976). Male Sprague-Dawley rats were pretreated with saline (the control group), apomorphine (2 mg/kg i.p.), or clonidine (350 µg/kg i.p.). Apomorphine was used as a reference standard. Fifteen minutes after pretreatment, animals received GBL(750 mg/kg i.p.) or saline (control animals). Twenty minutes after pretreatment, all animals received an aromatic amino acid decarboxylase inhibitor, NSD1015 (100 mg/kg i.p.). Fifty minutes after pretreatment, animals were sacrificed and brains were removed rapidly. Striata were isolated, then frozen at -70°C until deproteinized with 0.4 N perchloric acid. Tissue catcholamines were extracted with micropipette tips packed with glass wool to hold 20 mg of activated alumina. Catecholamines were analyzed by liquid chromatography electrochemical methodology using a 5 micron C_{18} reverse-phase column (25 cm x 0.46 cm) and eluting with a 0.1 M NaH_2PO_4 buffer (pH 3.2) containing 0.5 mM heptane sulfonic acid and 0.1 mM Na_2EDTA. The electrochemical detector oxidizing potential was 0.7 volts and the sensitivity was 20 nA/V. A summary of the experimental data is presented in following table:

Group	n	Striatal dopa- ng/g (X̄ ± SEM)	% Inhibition of GBL-induced accumulation of dopa
Saline+Saline+NSD	6	1523 ± 232*	—
Saline+GBL+NSD	6	4835 ± 351	0
Apomorphine+GBL+NSD	5	1846 ± 552*	90
Clonidine+GBL+NSD	5	1470 ± 260*	100

*Significantly different from GBL group ($p<0.05$). One-way Analysis of Variance and Duncan's Multiple Comparison Test.

For comparison, additional experiments were conducted with other putative dopamine autoreceptor agonists (e.g. TL-99 and 3-PPP). TL-99 produced a 100% inhibition of dopa accumulation at a dose of 5 mg/kg i.p. while 3-PPP produced a 73% inhibition of dopa accumulation at a dose of 10 mg/kg i.p. These data suggest that α-adrenoreceptor agonists may regulate striatal dopamine synthesis. The mechanism of α-adrenoreceptor regulation of striatal dopamine neurons remains to be elucidated, since clonidine may interact with dopamine autoreceptors, and/or pre-or postsynaptic α_2-adrenoreceptors.

128.24 EFFECTS OF DOPAMINE AUTORECEPTOR AGONISTS AT α_2-ADRENOCEPTORS. J.J. Welch, H.S. Kim*, A. Braunwalder*, G. Stone*, P. Loo* and J.M. Liebman. Res. & Dev. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

The novel agents, 3-(3-hydroxyphenyl)-N-n-propylpiperidine (3-PPP) and 6,7-dihydroxy-2-dimethylaminotetralin (TL-99), as well as low doses of apomorphine (APO), have been claimed to act selectively at dopamine (DA) autoreceptors (Hjorth et al., Life Sci. 28:1225, 1981; Goodale et al., Sci. 210:1141, 1980). The ability of these agents to cause certain behavioral effects in animals, in particular reduction of motor activity, has been attributed to their DA autoreceptor activity. Alternatively, Sumners et al. have contended that APO (Neuropharm. 20:1203, 1981) and some aminotetralin analogs of TL-99 (Eur. J. Pharm. 70:541, 1981) reduce motor activity by interacting with α_2-adrenoceptors in a manner similar to that described for clonidine. Using in vitro radioligand binding techniques and in vivo electrophysiological single cell recordings we have examined in more detail the interactions of these agents with α_2-adrenoceptors.

In receptor binding assays, the test agent's displacement of specific binding of ^{3}H-clonidine was measured in membrane preparations of bovine cerebral cortex. IC_{50} (µM) values for APO, 3-PPP and TL-99 were 0.2, 1.0 and 0.03, respectively. Our electrophysiological experiments were performed in chloral hydrate anesthetized rats. Extracellular single cell recordings were obtained from neurons within the substantia nigra zona compacta (SNC) or locus coeruleus (LC). Intravenous treatment with either APO, TL-99 or 3-PPP caused a marked suppression in SNC firing rate. However, at comparable doses, only TL-99 was effective in suppressing LC activity. This inhibition could be reversed by yohimbine but not haloperidol. 3-PPP partially inhibited firing in LC neurons but at doses which were 5 to 10 times higher than those required to inhibit SNC firing rate. APO never inhibited LC firing and, in fact, increased firing rate at doses above 0.5 mg/kg.

Taken together these results suggest that the pharmacological effects of TL-99 may not be attributed solely to its DA autoreceptor properties. Our tests show TL-99 to interact with α_2-adrenoceptors in vitro and in vivo, a feature which is generally characteristic for this class of compounds (Rusterholz et al., Eur. J. Pharm. 65:201, 1980). Therefore, the reduced motor activity seen in behavioral tests following TL-99 treatment could result from combined activation of DA and norepinephrine autoreceptors. In comparison with TL-99, APO and 3-PPP lacked affinity for α_2-adrenoceptors, supporting the notion that the behavioral effects of 3-PPP and low doses of APO are mediated primarily via DA autoreceptors. The presence of concurrent α_2-adrenoceptor agonism may alter in important ways the therapeutic profile of putative DA autoreceptor agonists.

128.25 THE DOPAMINE AUTORECEPTOR AGONISTS, TL-99 AND 3-PPP, ATTENUATE SELF-STIMULATION BUT NOT AVOIDANCE RESPONDING IN RATS. J.M. Liebman, H.M. Fenton*, S. Gerhardt* and L. Noreika*. Res. & Dev. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

The recently identified dopamine (DA) autoreceptor agonists, TL-99 (Goodale et al., Sci. 210:1141, 1980) and 3-PPP (Hjorth et al., Life Sci. 28:1225, 1981) exemplify a novel class of psychoactive drugs that may have potential for the treatment of schizophrenia and/or extrapyramidal motor disorders. These agents decrease impulse flow in striatal DA neurons as well as synthesis of DA in terminals. Using animal operant behavioral models that readily detect neuroleptics (postsynaptic dopamine antagonists), we have evaluated the degree of behavioral equivalence between neuroleptics and dopamine autoreceptor agonists.

All known neuroleptics impair rat Sidman avoidance performance, and this effect has been considered as a preclinical predictor of antipsychotic activity. In marked contrast, this behavior was unaffected by doses of TL-99 as high as 10 mg/kg i.p. or s.c. and by doses of 3-PPP up to and including 10 mg/kg i.p. and 30 mg/kg s.c. or p.o. These results suggest that either (a) TL-99 and 3-PPP will fail to show antipsychotic activity or (b) avoidance blockade is not essential for the prediction of antipsychotic efficacy.

The effects of TL-99 and 3-PPP were examined on lateral hypothalamic intracranial self-stimulation (ICSS) responding, which hypothetically assesses the "anhedonic" (reward-decreasing) impact of neuroleptic treatment. In a continuous reinforcement, bar-pressing task, both TL-99 (0.3-10 mg/kg i.p. or s.c.) and 3-PPP (0.3-10 mg/kg s.c.) markedly reduced response rates, but the dose-response slopes were shallow by comparison with those produced by haloperidol (0.1-0.3 mg/kg i.p.). No tolerance to the effects of TL-99 occurred over a 4 day treatment period. Additional experiments evaluated the possible contribution of nonspecific performance deficit to this effect on ICSS. In a shuttlebox test, latencies to initiate stimulation were preferentially evaluated by TL-99 (3-10 mg/kg i.p.), indicating decreased reward. The pattern of response decrement produced by TL-99 in bar-pressing ICSS was also assessed to determine whether an "extinction"-like effect was present. In common with haloperidol, a neuroleptic, and clonidine, an alpha-2 adrenoceptor agonist, TL-99 preferentially attenuated responding as the session progressed, suggesting such an effect. To determine whether the alpha-2 adrenoceptor agonist properties of TL-99 (Welch et al., this meeting) could mediate its effects on ICSS, the ability of yohimbine to antagonize TL-99 was examined. No such antagonism could be demonstrated. Taken together, these experiments indicate that the effects of TL-99 and 3-PPP on ICSS reflect, at least in part, attenuation of reward.

128.26 ATYPICAL NEUROLEPTICS INCREASE SELF-ADMINISTRATION OF COCAINE: A POSSIBLE SCREENING PROCEDURE FOR ANTIPSYCHOTIC DRUGS, D. C. S. Roberts and G. Vickers*. Dept. of Psychology, Carleton University, Ottawa, Ontario, Canada, K1S 5B6.

Classical neuroleptics have a variety of behavioral effects in rats which presumably are related to their ability to block dopamine (DA) receptors. These effects include attenuation of spontaneous and amphetamine-induced locomotor activity, induction of catalepsy, inhibition of apomorphine stereotyped behavior and disruption of conditioned avoidance responding. These behavioral effects have therefore been suggested as useful screening procedures for evaluating the possible antipsychotic effects of new drugs.

The last ten years have seen the development of several new antipsychotic agents which differ from classical neuroleptics in that they are less likely to produce extrapyramidal side effects or central depression. These "atypical" neuroleptics also display different profiles with regard to catalepsy and anti-amphetamine and anti-apomorphine properties. It is therefore likely that many of the former screens are more predictive of extrapyramidal side effects rather than antipsychotic potencies.

In animals trained to self-administer psychomotor stimulants, low doses of classical neuroleptics have been shown to increase drug intake. This study was undertaken to determine if "atypical" drugs would also increase self-administration rate and, if so, whether the order of potencies parallel their antipsychotic effects.

Male Wistar rats were trained to self-administer cocaine (1.5 mg/kg) on a CRF schedule for 4 hr/day. After a stable baseline was established, animals were pretreated with test doses of various neuroleptics. The dose required to increase cocaine intake by 25% were determined to be: sulpiride (12.0 mg/kg), metoclopramide (1.25), thioridazine (5.0) and for the classical neuroleptics: chlorpromazine (1.2) and pimozide (0.125). These potencies compare favourably to daily clinical dose, and suggest that this procedure may be a useful screen for neuroleptic activity.

There may be several reasons why neuroleptics increase cocaine intake. One suggestion is that they cause a partial blockade of the rewarding properties, and the increased intake represents a compensatory response. If this is the case, then the present data indicate that the mechanisms underlying psychomotor reward may be identical to those involved in psychotic behavior. (Supported by grants from M.R.C and N.S.E.R.C.).

128.27 ANTIPSYCHOTIC DRUGS: CATALEPTOGENIC POTENCY IS INVERSELY RELATED TO AN ACCELERATION OF NEURONAL ACTIVITY IN THE AMYGDALOID COMPLEX. Joel Gelman*, Kevin D. Alloway and George V. Rebec. (SPON: T. R. Bashore). Dept. of Psychol., Indiana Univ., Bloomington, IN 47405.

Antipsychotic drugs differ markedly in their ability to elicit extrapyramidal side effects which in the rat are manifest as cataleptic behaviors. Haloperidol, for example, produces catalepsy at relatively low doses, whereas clozapine, even at high doses, fails to elicit this response. We have previously shown that these behavioral differences are not reflected in the firing pattern of neurons in the neostriatum or nucleus accumbens (Rebec et al., Neuropharmacology, 1980, 19:281). In fact, haloperidol (2.0 mg/kg) and clozapine (20.0 mg/kg) produce a comparable increase in firing rate in both sites. Neurons in the amygdaloid complex, however, typically increase their firing rate to clozapine but fail to respond to haloperidol (Rebec et al., Pharmac. Biochem. Behav., 1981, 14:49). It is conceivable, therefore, that the cataleptogenic potency of the antipsychotic drugs is inversely related to their effects on amygdaloid activity.

To examine this hypothesis in more detail, we recorded neuronal activity in response to a wide range of antipsychotic drugs known to elicit different degrees of catalepsy. Tungsten microelectrodes were lowered bilaterally into the amygdaloid complex of adult, male rats. Each animal received only one injection to avoid residual drug effects. Our results indicate that over a broad dose range amygdaloid neurons increase their firing rate selectively to non-cataleptogenic antipsychotics. Thus, neurons throughout the amygdaloid complex were unresponsive to cataleptogenic drugs like haloperidol (0.5 - 20.0 mg/kg) and pimozide (1.0 - 4.0 mg/kg), whereas clozapine (5.0 - 20.0 mg/kg) and thioridazine (5.0 - 20.0 mg/kg), which do not produce catalepsy, typically accelerated amygdaloid activity. Interestingly, chlorpromazine (5.0 - 20.0 mg/kg), which is classified as a mild cataleptic agent, produced an intermediate response; approximately half the neurons in this group responded with a slight increase in firing rate. Our results support the view that the ability of antipsychotic drugs to elicit catalepsy is mediated, at least in part, by their effect on neuronal activity in the amygdaloid complex.

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128.28 SELECTIVE SUPPRESSION OF KAINATE-INDUCED ACTIVATION: A NON-GABA MEDIATED EFFECT, SPECIFIC TO BENZODIAZEPINES. G. de Bonnel*, D. Tardif* and C. de Montigny, Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec H3C 3J7.

Low doses of benzodiazepines (BZD) antagonize kainate (KA)-induced activation of CA_1 hippocampal pyramidal neurons. This effect is selective for KA since glutamate (GLU) and acetylcholine (ACh)-induced activations are much less affected (de Bonnel and de Montigny, Neurosci. Abst., 7:314, 1981). The present studies were carried out to determine (1) if this effect is mediated through a GABAergic mechanism, (2) if it is specific to BZD and (3) if there is a regional selectivity for this effect of BZD.

All experiments were conducted in urethane-anesthetized male Sprague Dawley rats (250-350 g). Five-barelled micropipettes were used for unitary recording and microiontophoresis of KA (1 or 3 mM in NaCl 0.4 M; pH: 8), GLU.HCl (0.1 M; pH: 8), ACh chloride (20 mM in NaCl 0.2 M; pH: 4), chlordiazepoxide.HCl (10 mM in NaCl 0.02 M; pH: 3) and GABA (0.1 M in NaCl 50 mM; pH: 4).

In a first series of experiments, microiontophoretic applications of GABA (6 nA) reduced the activations of CA_1 pyramidal cells by KA, GLU and ACh to a similar extent. Furthermore, low current applications of chlordiazepoxide (2 nA) decreased by 40% the excitatory effect of KA but failed to potentiate the action of GABA on ACh-activated cells. These data indicate that the selective effect of BZD on KA activation is not mediated through a GABAergic mechanism.

In a second series, lorazepam (LOR) (0.35 mg/kg, i.v.) injected during the activation of CA_1 pyramidal neurons by KA produced a 65% reduction of their firing rate, whereas chlorpromazine (4 mg/kg, i.v.), a sedative neuroleptic, and phenobarbital (10 mg/kg, i.v.), an anticonvulsant barbiturate, both failed to exert any consistent effect. Furthermore, pretreatment with RO 15-1788 (3.5 mg/kg, i.v.), a BZD antagonist, reduced by 60% the effect of LOR on KA activation. These results suggest that the antagonism of KA-induced activation is specific to BZD.

In a third series, we compared the effects of LOR (0.5 mg/kg, i.v.) in parietal cortex, CA_1 and CA_3 hippocampal regions. LOR induced an immediate and long lasting reduction of KA activation of 82% in CA_1 and of 25% in CA_3. In contrast, in parietal cortex LOR exerted only a transient suppression of KA activation.

The present results show that the selective antagonism of KA activation by BZD is not mediated through GABA. This effect of BZD might be related to their anxiolytic activity since (1) it is obtained with low doses, (2) it is long lasting, (3) it is not produced by sedative and anticonvulsant drugs, (4) it appears to be exerted selectively in the limbic system.

Supported by Canadian Medical Research Council grant MA-6444.

128.29 PRELIMINARY BEHAVIORAL AND PHARMACOLOGICAL STUDIES ON THE HALOPERIDOL METABOLITE REDUCED HALOPERIDOL. J. L. Browning*. P. B. Silverman, C. A. Harrington*, and C. M. Davis*. Sections of Analytical Neurochemistry and Neuropharmacology, Texas Research Institute of Mental Sciences, Houston, TX 77030 (SPON:J. Claghorn)

Previous reports indicate that the haloperidol (H) metabolite, reduced haloperidol (RH) should be about 5-25% as potent as H. We have found that patients with a high RH to H ratio, generally appear non responsive to H treatment. We have, therefore, begun investigating some of the behavioral effects and pharmacology of RH. Using inhibition of apomorphine-induced stereotypy in rats as as test for dopamine antagonist activity, we observed RH to be about 25% as active as H, similar to the observation of others. Displacement of ^{3}H-haloperidol by RH in calf caudate receptor preparations was 1/5 that of H, similar to its activity in the apomorphine-stereotypy test. Blood levels of H and RH at 1 hr. post i.p. injection in rat were 24±4 ng/ml (n=4) and 25±8 ng/ml (n=4) respectively. Rats were injected with 1 mg/kg/day i.p. for six days with saline, haloperidol, or reduced haloperidol. Twenty-four hours after last injection the blood levels of the haloperidol treated rats were -0.1±0.1 ng/ml haloperidol and 1.4±0.3 ng/ml reduced haloperidol, while blood levels of the reduced haloperidol treated rats were -0.3±0.3 haloperidol and 8.5±3.0 reduced haloperidol. These results indicate that there is little conversion of haloperidol to reduced haloperidol or of reduced haloperidol to haloperidol when administered i.p. Additionally, reduced haloperidol is eliminated from the blood much slower than haloperidol. Low doses (25 or 100 µg/kg)of RH had no appreciable effect on spontaneous motor activity. We looked at the effect of acute H and RH on weight gain in rats trained to consume their daily food intake in a 2 hr. ad lib exposure to rat chow (water was always available). Rats were treated with H (0.27 mg/kg) or RH (0.13 or 0.54 mg/kg) one hour prior to the feeding test. H-treated rats gained significantly less weight than in the preceeding control session. High dose RH-treated rats showed a lesser, but still significant, decrement in weight gain while no appreciable effect was noted following the low dose of RH. In addition platelet MAO-B was inhibited 50% by haloperidol at $1x10^{-5}$ M and reduced haloperidol at $2.5x10^{-5}$ M. Our results indicate that there are significant differences between the biologic activities of H and RH. The basis for these differences may be related to the non-responsiveness of these patients treated with H that have a high RH/H ratio in their plasma.

128.30 MJ 13805-1: A POTENTIAL NONBENZODIAZEPINE ANXIOLYTIC. Michael S. Eison, Duncan P. Taylor, Leslie A. Riblet, James S. New*, Davis L. Temple Jr., and Joseph P. Yevich*. Preclinical CNS Research, Pharmaceutical Research and Development Division of the Bristol-Myers Company, Evansville,IN 47721.

MJ 13805-1, known chemically as 4,4-dimethyl-1-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-2,6-piperidinedione hydrochloride, has been identified as a potential nonbenzodiazepine anxiolytic. It is equipotent with diazepam in anticonflict testing, attenuating shock-induced suppression of licking in water-deprived rats (Vogel test) at doses as low as 1.0 mg/kg, p.o. It is also active in inhibiting shock-induced fighting in the mouse (ED_{50} = 50.0 mg/kg, p.o.), a species in which the drug is rapidly metabolized. Although it inhibits conditioned avoidance responding in the rat (ED_{50} = 53.0 mg/kg, p.o.), it does not block apomorphine-induced stereotypy or induce catalepsy. Rather, it reverses trifluoperazine-induced catalepsy at anxiolytically relevant doses. MJ 13805-1 is an anorectic agent in the rat at doses higher than those required for anticonflict and anticatalepsy activity. It does not interact with CNS depressants, nor does it exhibit muscle relaxant or anticonvulsant activity. *In vivo*, the compound does not interact with great potency with either the cholinergic or noradrenergic system; it offers no protection from physostigmine-, norepinephrine-, or yohimbine-induced lethality. It does, however, produce consistent contralateral turning in rats unilaterally lesioned in the medial forebrain bundle with 5,7-dihydroxytryptamine. *In vitro*, MJ 13805-1 is not active in [^{3}H]-spiperone, [^{3}H]N-propylnorapomorphine, [^{3}H]QNB, [^{3}H]WB-4101, or [^{3}H]benzodiazepine binding. These data suggest that MJ 13805-1 is a potential anxiolytic compound unrelated to the benzodizepines in either structure or pharmacologic profile.

128.31 PHARMACOLOGIC PROFILE OF A POTENTIAL ANTIPSYCHOTIC AGENT: MJ 13859-1. L. A. Riblet, Michael S. Eison, Duncan P. Taylor, D. L. Temple Jr., and J. P. Yevich*. Preclinical CNS Research, Pharmaceutical Research and Development Division of Bristol-Myers Company, Evansville, IN 47721.

MJ 13859-1, known chemically as 8-[4-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride, was identified as an antipsychotic candidate in a rat conditioned avoidance procedure and an *in vitro* ^{3}H-spiperone binding assay. Activity in both of these systems is highly predictive of antipsychotic potential. This prediction was further supported by the ability of MJ 13859-1 to inhibit apomorphine-induced stereotypy in the rat and amphetamine-induced stereotypy in the dog. Most agents that cause extrapyramidal side effects in man also induce catalepsy in rats at doses lower than those required for inhibition of conditioned avoidance responding or apomorphine-induced stereotypy. MJ 13859-1 exhibited weak cataleptogenic activity in the rat at doses 6 and 8 times those required for inhibition of apomorphine-induced stereotypy and conditioned avoidance responding. Moreover, it lacked anticholinergic activity as determined by ^{3}H-QNB binding studies and failure to prevent physostigmine-induced lethality. This suggests that MJ 13859-1 would have minimal potential to produce extrapyramidal side effects in man. The pharmacologic profile of MJ 13859-1 will be compared to clozapine (CZP), thioridazine (TDZ) and other neuroleptics.

oral ED_{50}'s in mg/kg or IC_{50}'s in nM

	MJ 13859-1	CZP	TDZ
Conditioned avoidance (rat)	10	24	126
Apomorphine stereotypy (rat)	13	49	280
^{3}H-Spiperone binding (in vitro)	8	440	80
^{3}H-QNB Binding (in vitro)	>10,000	91	106

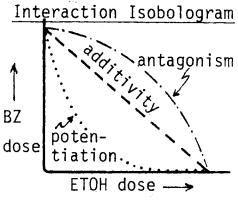
128.32 THE INTERACTION OF ETHANOL WITH DIAZEPAM OR LORAZEPAM. A. B. Davidson, S. Furman* and K. L. Keim. Pharmacology I, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

The interaction of ethanol [ETOH] with either diazepam [DZP] or lorazepam [LRZ] was studied in fasted 20 gm male CF1 mice. ETOH was given per os 20 to 30 min after oral administration of either benzodiazepine [BZ] and ED50 values were subsequently determined in two test procedures. Isobols were constructed by connecting those dose pairs which were equi-effective (ED50s) in producing the pharmacological effect (Loewe, S., Pharmac Rev 9: 237, 1957).

ED50 mg/kg po (each compound alone)

	LRR	TWD
DZP	253	2.1
LRZ	721	1.8
ETOH	5536	1826

Alone, LRZ was significantly weaker than DZP in producing loss of righting reflex [LRR; inability of a mouse to right itself for 10 min after being placed on its back]. When interacted with ETOH, the LRR-isobol for either DZP or LRZ deviated to below the additivity line indicating potentiation. That is, the combination of ETOH with either BZ resulted in a greater effect than that expected by the additive effect of the combined doses. Moreover, the area representing the LRZ + ETOH deviation from additivity (potentiation) was two-fold greater than that of DZP + ETOH.



DZP and LRZ alone were equipotent producing traction wire deficit [TWD; inability of a mouse to raise either hind paw to a 2 mm wire from which it was suspended by its forepaws]. However, in contrast to the LRR-isobol, the TWD-isobol suggests that the ETOH-interaction for either BZ is one of additivity.

Initial data indicate that while 100 mg/kg of the BZ antagonist Ro 15-1788 did not significantly modify the LRR produced by ETOH alone, it did antagonize the DZP potentiation of ETOH-induced LRR.

We demonstrate that the interaction of ETOH with another drug can vary with the intrinsic pharmacology of the drug as well as the tested endpoint.

128.33 PHARMACOLOGICAL ACTIVITIES IN RODENTS OF MINAPRINE : A NOVEL PSYCHOTROPIC DRUG. K. Bizière, J.P. Kan*, J.P. Muyard* and R. Roncucci*. Sanofi Recherche, Centre de Recherches Clin-Midy, 34082 Montpellier Cédex, France ; C.G. Wermuth*, Fac. Pharm. Univ. Louis Pasteur, Strasbourg, France.

Minaprine (M) {3-(2-morpholino)-ethylamino 4-methyl 6-phenyl pyridazine} is a new psychotropic drug with a therapeutic profile which differs from that of classical psychotropic agents. In man, M antagonizes "inhibitory states" which are characterised by decreased spontaneous activity, reduction in basic drives, slowed thoughts, feelings of tiredness and social withdrawal. M may also be beneficial in certain depressive states. In animal pharmacology M produced no remarkable change in the gross behavior of CDI mice for doses lower than 20 mg/kg i.p., at higher doses motility was slightly reduced. Like most antidepressants M antagonized reserpine-induced ptosis {ED_{50} = 5 mg/kg i.p. (4-7)}, reserpine-induced akinesia {ED_{50} = 8 mg/kg i.p. (6-11)}, and reserpine-induced hypothermia (threshold active dose = 5 mg/kg i.p.). In the behavioral despair test M reduced the duration of immobility, this effect exhibited a slow onset, maximal activity was reached 24 h after administration (threshold active dose 5 mg/kg i.p.). Unlike most antidepressants, M did not potentiate yohimbine-induced lethality in mice. In rats, M antagonized prochlorperazine-induced catalepsy {ED_{50} = 2 mg/kg i.p. (1-3)} and potentiated D-amphetamine induced stereotyped behavior (threshold active dose .75 mg/kg i.p.) M, 30 mg/kg i.p., 30 min after administration, slightly but significantly enhanced striatal DA levels (+ 12%) and decreased striatal DOPAC and HVA levels (- 46%). In vivo in mice, 1 h after administration M, 10 to 50 mg/kg i.p. significantly displaced (^{3}H)-spiperone binding to striatal membranes. In vitro, M did not affect either the release of the uptake of DA or (^{3}H)-spiperone binding to striatal membranes. M, 30 mg/kg i.p., 1 h after administration enhanced 5-HT levels in discrete brain regions including hypothalamus (+ 70%) and decreased 5-HIAA levels (- 50%). In vitro M did not modify the synaptosomal uptake of 5-HT and did not affect 5-HT release ; M had no affinity for type I 5-HT receptor labelled with (^{3}H) 5-HT. M, 30 mg/kg i.p., 30 min after administration, caused a widespread increase in ACh levels in various brain regions including brainstem, hipocampus, cortex and striatum (30 to 70% according to the region). Choline levels and acetylcholinesterase activity were not significantly modified by M in vitro and in vivo. Taken together these results may be relevant for the explanation of the therapeutic activities observed during the clinical evaluation of the drug.

128.34 DOPAMINE RADIORECEPTOR ASSAY AND GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF HALOPERIDOL IN HUMAN PLASMA. D.M.Martin*, E. Howard*, A.W.Caswell*, D.C. Bernot*, I.Extein, A.L.C.Pottash, M.S.Gold. Psychiatric Diagnostic Laboratories of America and Research Facilities, Fair Oaks Hospital, Summit, NJ.

Dopamine Radioreceptor Assays (RRA) for neuroleptic medications have received wide attention as they measure not parent compounds but all psychoactive metabolites that occupy dopamine receptors as well. We recently observed and reported a possible "therapeutic window" (5-15 ng/ml) in schizophrenic patients for haloperidol (HP) plasma levels as assayed by gas-liquid chromatography (GLC). Because HP has no significant active metabolites it is an ideal neuroleptic to compare GLC plasma levels and dopamine receptor occupancy as measured by a RRA. Plasma was drawn on eight (8) patients after 2-4 weeks on HP therapy and assayed for HP by GLC and dopamine occupancy by RRA. Eight (8) additional blank plasmas were spiked with HP at increasing concentrations and run under the same conditions as the patient samples. There was a significant ($r=.98$; $p < .001$) and linear relationship between the GLC and RRA data. These findings are consistent with the concept of a therapeutic "window" or "threshold" for haloperidol and demonstrate the relationship of RRA data to GLC haloperidol levels and hence to clinical response. The use of RRA for other neuroleptic medications must be studied further.

128.35 THE EFFECTS OF A BENZODIAZEPINE [BZ] AND OF NON-BZ ANXIOLYTICS ON MONKEY ELECTROCORTICOGRAM [ECoG] AND VARIABLE INTERVAL [VI] RESPONSE RATE. K. L. Keim and T. Smart*. Pharmacology I, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

Drug-specific frequency changes in the ECoG of unrestrained squirrel monkeys are characteristic of certain clinically active drug classes (Neurosci Abst 6: 366, 1980; 7: 526, 1981). We compared the effects of diazepam [DZP], phenobarbital [PHB], meprobamate [MPB], CL 218,872 [CL], tracazolate [TRZ], buspirone [BUS], and fenobam [FNB]. These agents have known efficacy and/or reported active as anxiolytics in animal models.

Drugs were administered I.G. 60 minutes prior to testing food-restricted monkeys on a 90 minute duration VI 60 second schedule of food reinforcement. Telemetered ECoG from anterior cortex was quantified and the 0-32 Hz range subdivided by computer into 8 bands for frequency analysis. Monkeys (3-5 per group) were given vehicle on Day 1 and a drug on Day 2, and the difference between frequency distributions and VI rates was used for comparative analysis.

DZP (0.5-8 mg/kg) reduced 4-8 Hz and increased 24-32 Hz ECoG activity and lever response rate; ataxia was seen at 8 mg/kg. PHB (10 & 20 mg/kg) decreased 4-16 Hz and increased 20-32 Hz waveforms; only 10 mg/kg increased VI rate but 40 mg/kg decreased response rate and caused sedation. MPB (10-80 mg/kg), like DZP, decreased slow and increased faster ECoG activity and response rate. Sedation was observed at 40 mg/kg MPB.

While CL increased lever rate, the ECoG profile was different. CL (40-160 mg/kg) increased 0-12 Hz and decreased 16-32 Hz cortical activity. TRZ (4 or 20 mg/kg) neither affected lever response rate nor did it alter distribution of 0-32 Hz ECoG frequencies.

BUS (2-16 mg/kg) reduced VI response rate, and tended to diminish 4-8 Hz with faster ECoG activity being unchanged; 8 mg/kg caused monkeys to cling to the cage side and 16 mg/kg caused ataxia, tremors, and prostration lasting several hours - effects characteristic of neuroleptic agents. FNB's ECoG profile can be summarized as amphetamine-like. That is, FNB (8 or 16 mg/kg) enhanced 2-12 Hz ECoG activity, disrupted VI responding, and caused agitation and disorientation with emesis.

The ECoG/operant task profile of the purported anxiolytics CL, TRZ, BUS and FNB differs from the more classic anxiolytic agents represented by DZP, PHB and MPB.

128.36 EFFECTS OF BUSPIRONE, AN ANXIOLYTIC DRUG, ON RAT BRAIN DOPAMINE METABOLISM AND FUNCTION. BRIAN A. McMILLEN AND CLAUDIA C. McDONALD*. Department of Pharmacology, University of Texas Health Science Center, Dallas, Texas 75235.

Buspirone is a clinically effective anti-anxiety drug. However, it does not displace diazepam binding, does not potentiate GABA in vivo (see Sanghera et al. these abstracts) and is not sedating or anti-convulsant. Buspirone inhibits dopamine (DA) autoreceptors without affecting postsynaptic DA receptors (McMillen et al. Neurosci. Abst. 1981). Since Bannon et al. (1981) demonstrated a lack of DA autoreceptors on the mesocortical DA projection, it was hypothesized that buspirone and molindone, an antipsychotic drug that preferentially inhibits DA autoreceptors (Alander et al. 1980), would selectively increase striatal DA metabolism. In contrast to haloperidol, which elevates striatal and frontal cortical DA metabolite concentrations, buspirone and molindone elicit little changes in frontal cortical DA metabolism at doses up to 3.0 mg/kg s.c. This dose of buspirone or molindone causes the same maximal response in striatum as 0.3 mg/kg haloperidol. Thus, the lack of autoreceptors in frontal cortex is of pharmacologic importance.

That preferential inhibition of striatal DA autoreceptors may reverse catalepsy by releasing extra DA to compete at postsynaptic receptors was tested by first inducing catalepsy with different drugs and then administering buspirone or molindone. Only buspirone (1.0 mg/kg s.c.) reverses catalepsy. This effect does not require presynaptic DA as catalepsy is reversed by buspirone in the DA depleted rat (2.0 mg/kg RO4-1284) as well as after postsynaptic DA receptor blockade by 1.0 mg/kg s.c. haloperidol or cis-flupenthixol. Small doses of apomorphine (30 or 100 µg/kg) fail to reverse catalepsy, which suggests buspirone does not act through the high affinity apomorphine binding site. The mechanism for reversal of catalepsy is unclear. The ability of buspirone to increase striatal DA metabolism is decreased 10 fold when buspirone is injected i.p. Thus, increased DA metabolism largely reflects direct antagonism by buspirone of DA autoreceptors. However, catalepsy reversal is unaltered by i.p. injection, which suggests that this effect of buspirone is due to both parent drug and its metabolite(s) at a site separate from DA receptors. Buspirone, which has fewer side-effects than other anti-anxiety drugs, may prove useful for treatment of extrapyramidal movement disorders involving rigidity (i.e. Parkinson's syndrome).
(Supported by USPHS MH-05831 and a contract from Mead/Johnson Pharmaceutical Division)

Alander, et al., J. Pharm. Pharmac. 32:780, 1980.
Bannon, Michaud and Roth, Mol. Pharmacol. 19:270, 1981.

128.37 ANXIOLYTIC DRUG EFFECTS ON LOCUS COERULEUS NEURONAL IMPULSE FLOW: BENZODIAZEPINES AND BUSPIRONE. M.K. Sanghera, B.A. McMillen, and D.C. German. Departments of Physiology, Psychiatry, and Pharmacology. University of Texas Health Science Center, Dallas, Texas 75235.

The locus coeruleus (LC) norepinephrine-containing cells have been hypothesized to play a role in anxiety. Treatments which decrease LC impulse flow are correlated with decreased levels of anxiety. Benzodiazepine (BZ) anxiolytic drugs, such as diazepam, decrease LC impulse flow. We have examined the effects of a non-BZ anxiolytic, buspirone, on dopaminergic neurons and found it to selectively block dopamine autoreceptors (McMillen, Matthews, Sanghera & German, J. Neurosci., in press). The present experiments were designed to determine whether BZs and buspirone commonly influence LC impulse flow.

Male rats (200-300 g) were anesthetized with chloral hydrate. Single LC cells were recorded from using single and multibarrel pipettes. Whole brain norepinephrine metabolism was examined by measuring MOPEG-SO_4 using a fluorometric assay. We found that: (1) unlike diazepam which decreases LC impulse flow (0.5 - 1.0 mg/kg i.v.) by an average of 40% (n = 8), buspirone (0.02 - 2.0 mg/kg i.v.) slightly increased LC impulse flow 20% (n = 14); (2) diazepam (1 mg/kg s.c.) significantly decreased MOPEG-SO_4 (14%), whereas buspirone (1 mg/kg s.c.) slightly increased MOPEG-SO_4 (11%); (3) whereas systemic or microiontophoresed BZ (diazepam and flurazepam, respectively) potentiated GABA inhibition of cerebellar Purkinje cells (7 of 11 cells), they did not potentiate GABA inhibition of LC impulse flow (7 of 7 cells); and (4) systemic and microiontophoresed buspirone neither potentiated GABA inhibition of Purkinje cells (3 of 3 cells) nor of LC cells (5 of 5 cells).

These data suggest that BZ anxiolytics and buspirone do not commonly influence LC impulse flow. Furthermore, although BZs are thought to act by enhancing GABA inhibition at BZ linked GABA receptors, they do not appear to decrease LC impulse flow by such a mechanism. As with in vitro binding studies, buspirone does not interact with BZs or GABA in vivo. Finally, the only known site of pharmacological activity for buspirone is on the dopamine system. Its mechanism for reducing anxiety is, as yet, unclear.

This research supported by Grants MH-30546 and MH-33513.

128.PO DOPAMINE RECEPTOR SUPERSENSITIVITY PREDICTS ANTIPSYCHOTIC RESPONSE TO Li_2CO_3 IN A BIOLOGICALLY DISTINCT SUBGROUP OF PSYCHOTIC PATIENTS. Jack Hirschowitz, Frank P. Zemaln and David L. Garver. Psychobiology Division, Univ. of Cin. School of Med., Cincinnati, OH 45267.

The present study demonstrates that a distinct subgroup of psychotic patients have a supersensitive growth hormone (GH) response to DA receptor agonist administration and that these patients show an antipsychotic response to Li treatment. This supersensitive GH response may be clinically used to select antipsycotic medication (Li_2CO_3 or conventional neuroleptics).

Blood samples from 50 patients with RDC schizophrenic symptoms and 10 normal controls were assayed for peak plasma GH response to administration of the DA receptor agonist, apomorphine (0.75 mg s.c.). Patients then entered a 2 wk Li trial with symptoms assessed with the serial New Haven Schizophrenic Index (NHSI).

Fifteen of the 50 patients demonstrated at least a 40% improvement in NHSI scores, were discharged on Li alone, and were designated Li-responders (Li-R). The mean peak GH resonse of the Li-R was 32.9±3.7 ng/ml, while the GH response of the 35 Li-nonR was 18.3±2.3 ng/ml, and that of the normal controls was 18.8±2.3 ng/ml, The GH response of the Li-R was significantly greater than that of the Li-nonR ($p<0.001$), the normal controls ($p=0.008$) or as we have previously reported patients with an affective disorder. The significantly elevated GH response elicited by a DA receptor agonist is consistent with the hypothesis of a DA receptor supersensitivity in Li responsive patients.

The present study suggests that Li responsive psychotic patients may be predicted from their GH response, and this information used to make medication decisions. Patients with an elevated GH response may be placed on Li, which has neither the parkinsonian side effects of neuroleptics, nor their long term risk of tardive diskinesia; while patients that do not show an elevated GH response may be placed directly on neuroleptics.

A GH response criteria that best discriminated between Li response/nonresponse was determined utilizing Information Theory with the method of Prior Probabilities and Bayesian Analysis. A peak GH $\geq$ 20 ng/ml was found to maximally reduce the uncertainty of predicting Li response/nonresponse. The GH test employing the 20 ng/ml criteria accurately predicted 87% of the Li-R in the present study (test sensitivity) while excluding 71% of the Li-nonR (test specificity). The 87% sensitivity and 71% specificity of the GH test compares favorably to other tests in clinical medicine. For example a liver scan for prediciting hepatic pathology has a sensitivity of 90% and a specificity of 63%, while a clinical exam plus mammography for prediciting breast carcinoma has a 67% sensitivity and a 98% specificity.

128.PO THE OUTPUT THEORY: A NEW HYPOTHESIS CONCERNING THE ROLE OF BETA ADRENERGIC RECEPTORS IN ANTIDEPRESSANT THERAPY AND ADAPTATION TO STRESS. E. A. Stone. Dept. Psychiatry, New York Univ. Sch. Med., New York, NY 10016

There are two current theories of catecholamine (CA) involvement in antidepressant therapy. One proposal assumes that antidepressant agents act by enhancing the actions of norepinephrine (NE) at its brain receptors (NE-release theory). The second assumes that these agents act by reducing the sensitivity of the brain beta adrenergic receptor coupled cAMP system (subsensitivity hypothesis). Both of these theories are in conflict with considerable data. For the NE release theory the major obstacle is the fact that there is a large discrepancy between the latency of onset of the pharmacological effects of antidepressants (several minutes) and their therapeutic effects (1-4 weeks). For the subsensitivity theory the major areas of disagreement include the findings that (a) beta adrenergic subsensitivity in several peripheral organs caused by chronic stress of CA injection does not lead to decreases but rather to increases in CA-stimulated organ output, (b) propranolol is not an effective antidepressant but rather induces depression, (c) thyroid hormones enhance responsiveness to NE and potentiate the therapeutic effects of tricyclic antidepressants, (d) depression is accompanied by an increased secretion of cortisol, a hormone that has permissive actions on NE-stimulated function, (e) chronic antidepressants may not produce decreases but rather increases in net noradrenergic neurotransmission and (f) cAMP may not be involved in central noradrenergic neurotransmission but probably subserves other metabolic effects of NE. To reconcile these discordant data we have proposed a new hypothesis, the output theory, which is derived from the latter two. The new theory assumes that depression or adverse behavioral effects of stress result when the output of brain cells bearing beta adrenergic receptors (beta effector cells) is too low to meet increased demand resulting from stress or other biologically disruptive events. According to this view antidepressants act by a mechanism akin to adaptation to chronic stress in which there is a prolonged increase in the availability of NE in the brain. The prolonged increase is necessary for NE to induce, via the beta adrenoceptor-cAMP system, trophic or long term metabolic effects which increase the output of beta effector cells to a level commensurate with demand. The desensitization of beta adrenergic receptors by antidepressants and stress, previously thought to reflect reduced neurotransmitter function, is now reinterpreted to be a process which either limits further trophic-metabolic effects or increases the efficiency of cellular output by reducing the level of input necessary for a given level of output. Supported in part by USPHS grants MH 22768 and MH 08618.

129.1 THE ROLE OF ALTERED AXOPLASMIC TRANSPORT OF NERVE GROWTH FACTOR IN CAPSAICIN-INDUCED SUBSTANCE P DEPLETION. M.S. Miller, I.G. Sipes*, S.H. Buck and T.F. Burks. Dept. of Pharmacology, Arizona Health Sciences Center, Tucson, AZ 85724.

Little is known regarding the factors responsible for regulating the substance P (SP) content of primary afferent neurons. SP is depleted from the sensory neurons of neonatal and adult animals which are exposed to antibodies to nerve growth factor (NGF). This suggests that sensory neurons must continually be exposed to NGF to maintain SP levels. Capsaicin (C), like exposure to antibodies to NGF, is also capable of depleting SP from primary afferent neurons. This suggests that alterations in the availability of NGF may be involved in the depletion of SP by capsaicin. The role that alterations in the retrograde axoplasmic transport of NGF may play in C-induced depletion of SP was the subject of this investigation. The dose-response relationship for depletion of SP from primary afferent neurons by C was investigated in Hartley guinea pigs (300-400 g). Animals were treated with C (2-50 mg/kg s.c.) and SP content of DRG was determined 1 day or 4 days after treatment by RIA. In addition, the effects of various doses C on the retrograde axoplasmic transport of iodinated mouse NGF were determined 1 day and 4 days after C treatment. C produced no detectable depletion of SP from DRG when measured 1 day after treatment. C depleted SP from dorsal root ganglia (DRG) in a dose-dependent manner when measured 4 days after C treatment. The threshold dose of C for SP depletion was approximately 4 mg/kg s.c. Maximum depletion of SP from DRG (approximately 80%) was seen after single doses of C as low as 10 mg/kg s.c. Retrograde axoplasmic transport of ^{125}I-NGF was inhibited in a dose-dependent manner when measured either 1 day or 4 days after C treatment. C appeared to inhibit the quantity of NGF transported and not the rate of transport. Doses of C which inhibited retrograde transport of NGF also depleted SP from DRG. Alterations in NGF transport preceeded SP depletion. The cause and effect relationship between depletion of SP and alterations in the retrograde axoplasmic transport of NGF was investigated in guinea pigs which were treated with C (10 mg/kg s.c.) and supplemented with mouse NGF for 4 days (1.0 mg/kg i.p.). Four days after C treatment SP content of DRG was assessed. Administration of NGF completely blocked C-induced depletion of SP. Administration of NGF alone produced no significant change in dorsal root ganglia substance P content. These data indicate that C-induced SP depletion in DRG may be a result of decreased availability of NGF to the cell bodies of sensory neurons due to alterations in retrograde axoplasmic transport. (Supported by USPHS grant no. NS 15420).

129.2 CHARACTERIZATION OF THE PEPTIDE NEUROTOXIC EFFECTS OF CAPSAICIN IN THE GUINEA PIG. S.H. Buck, J.H. Walsh*†, S.P. Duckles, T.P. Davis, H.I. Yamamura, and T.F. Burks. Dept. Pharmacology, Univ. Arizona Hlth. Sci. Ctr., Tucson, AZ 85724, †Dept. Medicine, Sch. of Medicine, UCLA, and †CURE, VA Center, Los Angeles, CA 90073.

Systemic administration of capsaicin (CAP), the active ingredient of hot peppers, in rats produces a depletion of substance P (SP) that is limited to primary afferent neurons. This depletion is accompanied in adult rats by a marked reduction in sensitivity to nociceptive chemical stimuli without substantial changes in sensitivity to nociceptive heat. CAP produces a similar depletion of primary afferent SP in adult guinea pigs but this is accompanied by a marked loss of heat sensitivity in addition to the loss of chemogenic sensitivity. Other sensory modalities in the guinea pig are not affected. We have now more fully characterized the effects of CAP on primary afferent SP in guinea pigs and investigated the effects on primary afferent levels of somatostatin (SOM), vasoactive intestinal polypeptide (VIP), and cholecystokinin (CCK).

Adult guinea pigs were administered CAP as a suspension subcutaneously. Tissues were extracted with 2 N acetic acid or boiling water (for CCK). SP was measured by RIA using an antiserum with cross-reactivity of 1% with physalaemin, 0.2% with SP-(2-11), SP-(3-11), SP-(4-11), and SP-(5-11), and less than 0.001% with each of 20 mammalian neuropeptides. SOM was measured using a commercial RIA kit (Immuno Nuclear Corp.) and previously characterized antisera were employed for the RIA determination of VIP and of CCK.

A single dose of 1 mg/kg CAP had no effect on SP levels in dorsal root ganglia (DRG) or dorsal spinal cord (DC). 5 mg/kg CAP depleted SP in DRG by 70% and in DC by 20%. 50 mg/kg CAP depleted SP in DRG by 85% and in DC by 40%. Higher doses produced no greater depletion. Maximal SP depletion in DRG and DC did not occur until 4 days after CAP injection. Supramaximal doses of CAP also depleted SP in superior cervical ganglia, cerebral and mesenteric arteries, and adrenal gland but not in ventral spinal cord (VC), hypothalamus, corpus striatum, or GI tract. 4 days after CAP, there was a lesser decrease in CCK in DRG and a decrease in VC but no change in SOM or VIP in DRG, DC, or VC. 10 days after CAP, there was maximal SP depletion in DRG and DC but no change in CCK, SOM, or VIP. 10 weeks after 50 mg/kg CAP, there was maximal SP depletion in DRG and DC but no change in SOM. At this last time point, the heat and chemogenic insensitivities were still present.

Our results indicate that the adult guinea pig is extremely sensitive to the effects of CAP and that the compound produces extraordinarily long-lasting biochemical and physiological changes in certain sensory neurons in this species. In addition, the specific sensory effects appear to result from an action of CAP that is specific for SP-containing primary afferent neurons. (Supported by USPHS and PMA, NIMH RSDA to HIY, NIH Predoc. Flwshp. to SHB)

129.3 SUBCLASSES OF PRIMARY SENSORY NEURONS AND AMINE OR PEPTIDE SECRETORY CELLS REVEALED BY SELECTIVE ACID PHOSPHATASE SUBSTRATES. J.Dodd*and T.M.Jessell.Department of Neurobiology Harvard Medical School, Boston, MA 02115.

A subpopulation of small-diameter sensory neurons associated with the processing of noxious peripheral stimuli contain the peptides substance P and somatostatin. The transmitter identity or peptide content of the remaining small sensory neurons is unknown, although many are characterized by the presence of an acid phosphatase isoenzyme. Previous studies on this isoenzyme have used substrates that are hydrolyzed by all acid phosphatases. We have found that thiamine monophosphate, ephedrine-O-phosphate and phosphorylcholine, which all contain a basic nitrogen and exhibit steric hindrance near the O-phosphate group, are selective substrates for the acid phosphatase isoenzyme within small sensory neurons.

Approximately 45% of neurons within rat dorsal root ganglia contain the isoenzyme and these cells exhibit little or no overlap with neurons containing substance P or somatostatin. Small neurons in the trigeminal ganglion also contain the isoenzyme. However, less than 3% of neurons in the nodose ganglion are stained and there is little differentiation in size between stained and unstained neurons. The central terminals of neurons containing the isoenzyme are restricted to lamina II of the dorsal horn. The isoenzyme is absent from all neurons that originate in the CNS.

In addition, the isoenzyme is present in a subclass of neurons in the superior cervical ganglion, with some evidence of a topographic localization. About 80% of cells in the adrenal medulla contain the isoenzyme. This population overlaps with epinephrine-containing cells, identified by phenylethanolamine-N-methyl transferase immunoreactivity. Pancreatic islet cells that contain insulin also exhibit isoenzyme staining but there is no apparent overlap with islet cells containing glucagon or somatostatin. In the thyroid gland, parafollicular cells that are known to contain calcitonin also express the isoenzyme.

Activity of the isoenzyme purified from dorsal root ganglia is highly pH dependent, with an optimum at pH 5.5 suggesting a localization in secretory granules. The isoenzyme is inhibited by p-chloromercuribenzoate and also by L(+)-tartrate. A subclass of small-diameter sensory neurons involved in processing cutaneous sensory information therefore exhibit biochemical properties common to amine and peptide secretory cells of neural crest origin or endocrine function.
Supported by The Dysautonomia Foundation, NIH grant NS1736901 and a Muscular Dystrophy Association Fellowship to J.D.

129.4 SP, BUT NOT SS, CCK OR VIP, IS A SENSORY TRANSMITTER CANDIDATE IN AN ELASMOBRANCH LACKING UNMYELINATED AFFERENTS. R.B. Leonard and T.C. Ritchie. Marine Biomedical Institute and the Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas.

Four neuropeptides, substance P (SP), somatostatin (SS), cholecystokinin (CCK) and vasoactive intestinal polypeptide (VIP), are considered to be candidates as neurotransmitters for small myelinated and unmyelinated primary afferents in mammals. SP and SS may be associated with the transmission of nociceptive input to the spinal cord, but on the available evidence it is not possible to relate the peptides to specific sensory or nociceptive submodalities. We have studied the origin and distribution of these peptides with immunohistochemistry (PAP) in the stingray, an elasmobranch which has virtually no unmyelinated primary afferents. Instead. there are both large and small afferent fibers,and they have dichotomous termination zones in the dorsal horn. We have previously reported that both SP- and SS-like immunoreactive neuronal elements are present in the stingray spinal cord. The highest density for both peptides is in the superficial aspect of the substantia gelatinosa (SG), and less dense immunoreactivity is scattered throughout the spinal gray matter. We observed, after multiple unilateral dorsal rhizotomies, a virtually total depletion of SP in the superficial dorsal horn, while no decrease was detected for SS. We now report that CCK- and VIP-like immunoreactive neuronal elements are also present in stingray spinal cord and their distributions bear striking resemblance to that of SS. In sections stained for CCK, VIP or SS, bundles of stained fibers extend medially from a prominent tract in the lateral funiculus to form a fiber plexus at the lateral aspect of the nucleus proprius. Bundles of fibers, as well as single fibers, then spread dorsally and medially through the SG. Like SS, CCK and VIP stained granules, probably representing terminals, are most dense in a thin band at the superficial margin of the SG. However, VIP stained granules are also distributed more ventrally in the SG. CCK as well as both SS and SP, were observed to be particularly dense in the lateral third of the SG. In our material, both CCK and VIP were more densely distributed throughout the deep dorsal horn and the ventral horn than either SS or SP. Immunoreactive cells were observed in the ventral horn, dorsal horn and around the central canal for SS, in the ventral horn for CCK, and at the ventral margin of the SG for VIP. After multiple unilateral dorsal rhizotomies, we detected no decrease in either CCK or VIP staining. In conclusion, our studies indicate that SP is a candidate neurotransmitter for small myelinated primary afferents in stingrays while the SS, CCK and VIP have a quite different origin.

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129.5 IMMUNOHISTOCHEMICAL EVIDENCE FOR COEXISTENCE OF CHOLECYSTOKININ- AND SUBSTANCE P-LIKE PEPTIDES IN PRIMARY SENSORY NEURONS. C.-J. Dalsgaard*, S. Vincent*, T. Hökfelt*, G.J. Dockray* and C. Cuello*. (SPON: M. Eriksson). Depts. of Anatomy and Histology, Karolinska Institutet, Stockholm, Sweden. Dept. of Physiology, Liverpool University, Liverpool and dept. of Pharmacology, Oxford University, Oxford, England.

From previous biochemical and immunohistochemical work it has been suggested that, in addition to substance P (SP), somatostatin and VIP, also cholecystokinin (CCK) may be present in primary sensory neurons. In the present study the occurrence of CCK- and SP-like immunoreactivity in the dorsal root ganglia and spinal cord of the rat was investigated using indirect immunohistochemistry.

Male albino rats (250 g) were anesthetized and colchicine (10 µl: 200 µg) was administered into the midthoracic subarachnoid space via an intrathecal polyethylene catheter. After 24h. postoperative survival the animals were perfused with 4% formalin and the mid-thoracic dorsal root ganglia and spinal cord were removed, cut on a cryostate at 14 µm and processed for indirect immunohistochemistry employing antisera to CCK-4 or SP. After photography of the dorsal root ganglion sections incubated with CCK-antiserum these sections were treated with acid potassium permanganate to elute the first antibody, restained with the SP-antiserum and reexamined. It was controlled that no immunoreactivity was present after the elution and that no crossreactivity occurred at the immunohistochemical level.

Several CCK-positive and SP-positive cell bodies were observed in the dorsal root ganglia and the restaining experiment revealed that all CCK-immunoreactive neurons also were SP-positive and vice versa. In the spinal cord CCK immunoreactive fibers and terminals were observed in the superficial layers of the dorsal horn where also a dense network of SP positive fibers and terminals could be seen.

The present findings give strong evidence for coexistence between CCK- and SP-like immunoreactivity in primary sensory neurons.

129.6 LEUCINE ENKEPHALIN AND SUBSTANCE-P IDENTIFIED IN TRIGEMINAL GANGLION NEURONS INNERVATING THE CORNEA IN THE CAT. C. Morgan, W.C. deGroat, P.J.Jannetta. (Spon:J.R.Boston).Depts.Neurol.Surgery and Pharmacology, Univ.Pitt. Sch. of Med., Pittsburgh, PA 15261

In previous HRP studies we have shown that the cornea is innervated by sensory axons whose cell bodies are located in the ophthalmic division of the trigeminal ganglion (Vg). While the cornea responds to many stimuli such as heat, cold, touch and pressure, pain is probably the most effective stimulus. Since both substance-P (SP) and leucine enkephalin (LENK) have been shown to be involved as possible neurotransmitters in pain pathways, it was decided to determine if they were contained within corneal afferent neurons in Vg.

The fluorescent dye True Blue was injected into the corneas of anesthetized adult cats. After 10-18 days the cats were perfused with saline and 4% paraformaldehyde. The Vg were frozen, cut into 28 µm sections, mounted on slides and processed for SP and L-ENK using the fluorescein isothiocyanate (FITC) immunohistochemical process. Because sections of each ganglia were processed for different peptides, cell counts reported are less than would be expected if entire ganglia were processed for only one peptide.

Both SP and LENK were identified in Vg neurons throughout the ganglion while neurons labeled with True Blue were located only within the ophthalmic division. Within the ophthalmic division SP was identified in an average of 106 cells per section while LENK was seen in 20 cells per section and True Blue was found in 27 cells per section. A small number of True Blue-labeled corneal neurons also contained peptides. 36% contained SP and 14% contained LENK.

The identification of SP in all three divisions of the Vg and especially in the corneal afferents is not surprising in view of the trigeminal nerve's role in pain function. However, the presence of LENK, not commonly thought of as a transmitter in sensory neurons raises the possibility that these neurons may have an autonomic rather than sensory function. The low percentage of neurons containing SP or LENK may reflect actual numbers of peptide-containing neurons or may result from concentrations of peptides which are below the sensitivity of our methods. Possibly higher numbers might be obtained by using colchicine. The larger percentage of True Blue neurons not containing SP or LENK suggest two possibilities: first, they may contain other transmitters and are involved in non-noxious sensation, or second, they are involved in noxious sensation but through transmitters other than SP or LENK. Finally, since SP & LENK are contained in neurons whose axons project to the cornea, it seems likely that these peptides should also be contained in the cornea. Studies are presently underway to demonstrate this.

129.7 PIA ARACHNOID CONTAINS SUBSTANCE P PROJECTING FROM TRIGEMINAL NEURONS: IMPLICATIONS FOR VASCULAR AFFERENT NEUROTRANSMISSION. L.Y. Liu-Chen, Dae H. Han*, M.A. Moskowitz, Division of Neural and Endocrine Regulation, Department of Nutrition and Food Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139., Neurosurgery Service, Massachusetts General Hospital, Boston, MA 02114.

Substance P (SP) is present in measurable amounts in pia arachnoid (PA) overlying forebrain from rat, cat, dog, and calf. Levels of the immunoreactive SP (ISP) are comparable to those found in other peripheral structures receiving innervation from dorsal root or trigeminal ganglia, but are lower than those measured in the trigeminal nerves, dorsal horn of spinal cord, or substantia nigra. When subjected to reverse phase HPLC separation and analysis by RIA, the acid extract of bovine PA contained a single peak of immunoreactivity with a retention time which corresponded to that of authentic SP. To determine the origin of ISP in cat pia arachnoid, unilateral trigeminal ganglionectomies were performed in thirteen cats. The corneal reflex was absent on the lesioned side in all cats as was the response to pin prick response in all three divisions. Animals were sacrificed as early as 19 days and as late as 55 days after surgery. ISP levels in PA were reduced from 73.7 ± 14.0 fmole/mg protein on the lesioned side to 34.0 ± 7.6 fmole/mg protein on the lesioned side (mean ± s.e.m., $P < 0.001$). The ipsilateral depletion of SP in the last 5 operated cats was greater than 75%. Corneal ISP levels decreased significantly ipsilaterally, which is consistent with published results. To assess whether sympathetic nervous system contributes to ISP in PA, bilateral superior cervical ganglionectomies were performed in rats. Levels of ISP in PA were 22.5 ± 4.6 in controls versus 27.7 ± 5.8 fmole/mg protein, in lesioned animals. Intraventricular injections of 6- hydroxydopamine in rats did not change ISP levels in PA. Hence most SP resides within projections from ipsilateral trigeminal ganglion. The identification of SP within such a trigeminal pathway suggests new strategies for prophylaxis and treatment of unilateral vascular head pains associated with strokes and certain types of migraine headaches.

129.8 IN VITRO RELEASE OF SUBSTANCE P-LIKE IMMUNOREACTIVITY FROM PUTATIVE AFFERENT NERVE ENDINGS IN BOVINE PIA ARACHNOID. M.A. MOSKOWITZ, M. BRODY*, L.Y.LIU-CHEN. Stroke Research Laboratory, Massachusetts General Hospital, BOSTON, MA 02114

Trigeminal neurons provide the major source of isubstance P in pia arachnoid (75%). The majority of these neurons are perivascular in location and project from the ophthalmic division of the trigeminal ganglia.

Studies were undertaken to examine the release of substance P from pia arachnoidal nerve endings by drugs or ions. 2-3 gms of tissue with attached blood vessels were placed in a Swin-Lok chamber (Nuclepore, Pleasanton,CA) and superfused with Krebs bicarbonate buffer or isosmotic buffers containing high potassium or capsaicin. Substance P was released following exposure to high potassium,51 or 100 mM; release exceeded basal levels by as much as 135 or 220% respectively ($p < .001$ for either treatment). This increase was observed within 6 hours after infusing high potassium and remained elevated during the entire 24 minute high K^+ superfusion. iSP release returned quickly to basal levels either upon or shortly after lowering the potassium concentration. The time for half maximum release was 14.2 and 9.8 minutes, during perfusion with 51 and 100 mM potassium, respectively, as compared to 38 minutes for 3.5 potassium. The amount of substance P released during basal or stimulated state differed from one experiment to the next. The means of the evoked release constants (percent endogenous release/minute) ranged between 0.026-0.056 during the basal periods and increased to 0.056-0.116 during superfusion with 51 mm potassium.

Capsaicin evoked the release of SP from pia arachnoid when added to Krebs bicarbonate at concentrations above 10^{-8}M. Capsaicin induced release was calcium and concentration dependent. A second pulse of capsaicin evoked SP release as did a second pulse of high potassium (100mM). Capsaicin also stimulated SP release when added after prior stimulation with potassium. Evoked release by potassium was also calcium dependent and abolished by removing calcium and adding EGTA.

Fractional SP release in pia arachnoid appeared significantly less than the release reported previously from substantia nigra, hypothalamus, or striatum; however, it is comparable to that amount previously reported for dorsal horn of spinal cord. The apparant reduced fractional release may reflect a higher proportion of axons to terminals in this tissue. Release of substance P, a vasodilating peptide from perivascular nerve endings may alter tone and reactivity of pial blood vessels when these neurons become depolarized.

129.9 FELINE TRIGEMINAL PROJECTIONS TO PIA ARACHNOID AND DURA. M.R. MAYBERG*, N.T. ZERVAS, AND M.A. MOSKOWITZ. STROKE RESEARCH LAB. MASSACHUSETTS GENERAL HOSPITAL, BOSTON, MA 02114.

Horseradish peroxidase studies were performed to identify a trigeminal projection to supratentorial dural and large pial vessels in the cat. HRP (Sigma type VI, 2 mg/cc) was impregnated in polyvinyl alcohol (PVA) and ethylene vinyl alcohol copolymer (EVA) to restrict diffusion away from the injection site and applied onto: (1) proximal right middle cerebral artery (MCA) (N=11), (2) anterior superior sagittal sinus (N=3), (3) posterior superior sagittal sinus (N=3), (4) right lateral sinus (N=3), (5) 1 cm sq. over right convexity removed from large vessels (N=2). After 72 hours, the animals were perfused with 2.5% glutaraldehyde in 0.1 M phosphate buffer and both trigeminal, geniculate (VII) superior vagal (X), glossopharyngeal (IX), and superior cervical ganglia (SCG) were removed, sectioned frozen, and processed by the TMB method. Reaction product was observed in ipsilateral trigeminal ganglion cells in every animal when HRP was applied to middle cerebral artery and right lateral sinus, and bilaterally for anterior superior sagittal sinus and posterior superior sagittal sinus. The number of positive cells varied from 3 to 180 per animal, ranged in size from 15 to 60 microns, and were distributed throughout the first division of the ganglion. The SCG showed HRP-positive cells bilaterally (more prominently ipsilaterally) in every animal, with from 1 to 50 cells per animal located diffusely throughout. No tracer was present in geniculate, vagal, or glossopharyngeal neurons. No reaction product was observed in any ganglion when HRP was: (1) applied to the convexity removed from large pial vessels, (2) applied to the MCA with proximal ligation of the artery, or (3) infused intravenously. These findings show the existence of connections between pial vessels and dura to trigeminal ganglia. Such connections may be important in neurotransmission of afferent information (perhaps of a nociceptive nature) from intracranial pial and dural vessels.

129.10 ELECTROCHEMICAL MONITORING OF ENDOGENOUS NEUROTRANSMITTER RELEASE FROM RAT THALAMIC SLICES. [Margaret E. Rice* and Ralph N. Adams] (SPON: A. F. Oke). Dept. of Chem., Univ. of Kansas, Lawrence, KS 66045.

Neurotransmitter release from sensory processing areas during various peripheral stimulations can be readily monitored using in vivo chronoamperometry. One hindrance in such measurements has been the limited selectivity of the electrochemical probe. At an applied potential of +0.5 V vs. Ag/AgCl in the thalamus, one can expect concurrent oxidation of norepinephrine, serotonin, and their acid metabolites, as well as high levels of ascorbic acid.

Brain slices can readily be used as a model for this system to address the problem of specificity. Using an electrochemical electrode coated with ascorbic acid oxidase, ascorbic acid is eliminated from the electrochemical signal. Using this probe, neurotransmitter release has been monitored following tissue stimulation by such substances as potassium, veratridine and acetylcholine.

This technique has the major advantage of investigating the release of endogenous species rather than introduced radiolabeled compounds.

129.11 NEUROTRANSMITTERS IN THE ELECTROSENSORY SYSTEM OF GYMNOTID FISH. L. Maler, H. Kaplan* and B. Mathieson*. Dept. of Anat., Univ. of Ottawa, Ont. K1N 9A9, Canada.

The posterior lateral line lobe (PLLL) of gymnotid fish is a laminated structure receiving a direct topographic input from electroreceptor afferents (Maler et al., '74). Primary afferent input, descending input, and intrinsic fiber plexuses all terminate in different laminae (Maler, '79; Maler et al., '81). The simple and well characterized structure of the PLLL make it a favourable model for basic studies of neurotransmitter function. We have therefore undertaken to determine the transmitter(s) associated with various fiber systems in the PLLL.

Previous work demonstrated a cholinergic input to the dorsal molecular layer of the PLLL (Maler et al., '81). We have recently used ^{3}H-QNB to demonstrate that the cholinergic receptor is muscarinic in nature and that it is confined to the dorsal molecular layer as well; no nicotinic (αBuTx binding) receptors were found. We have used microdensylation methods to determine the level of various amino acids (β-alanine, aspartate, GABA, glutamate, glycine) in the whole PLLL as well as in individual laminae. Glutamate was found to be far greater in the dorsal molecular layer (100nmoles/mg) than in the whole PLLL (50nmoles/mg); since the main input to this layer is cerebellar parallel fibers (Maler et al., '74) this is consistent with the hypothesis that these fibers are glutaminergic. Aspartate levels are enhanced in the ventral molecular layer.

GABA and glycine are found in all laminae. Autoradiography was used to show that GABA is taken up by specific neuronal types (polymorphic cells, stellate cells, neuron of the v.mol.l.) whereas glycine uptake is diffuse. In addition immunohistochemistry was used to show that the same cell types that take up GABA also contain GAD (the antiserum to teleost GAD was supplied by Drs. Su and Wu). GABA is thus likely to be a transmitter whereas glycine is not.

This work was supported by the Medical Research Council of Canada.

130.1 CLEAVAGE OF ENKEPHALINS FROM PEPTIDE E BY CHROMAFFIN GRANULE ENZYMES C. M. Troy* and J. M. Musacchio. Dept. of Pharmocology, New York Univ. Med. Ctr. New York 10016

Coexistence of enkephalins and their precursors within bovine chromaffin granules indicates that the processing enzymes could also be present in the secretory granules. We have partially purified and characterized enzyme activities in the chromaffin granules which cleave enkephalins from larger peptides.

Chromaffin granules were isolated from bovine adrenal medullae by differential centrifugation. Enzyme activity was detected in the membranous and soluble components of the granules; radioiodinated peptide E was utilized as the substrate. Peptide E is a 3200 dalton adrenal peptide identified and sequenced by Kilpatrick et al (PNAS 78: 3265-3268, 1981). Cleavage of [^{125}I]-peptide E by trypsin yielded [^{125}I]-Leu-E and [^{125}I]-Met-E-Arg^{6}; further processing with carboxypeptidase B converted [^{125}I]-Met-E-Arg^{6} to [^{125}I]-Met-E. Digestion products were separated and identified by HPLC on an Altex RP-18 column; peptide identities were confirmed by TLC of the HPLC fractions, autoradiography was used to visualize the iodopeptides after TLC. The TLC-autoradiography system also allowed rapid analysis of large numbers of digests. Chromaffin granules were lysed in hypotonic buffer at pH 5.7, membranous and soluble constituents were separated by centrifugation and each incubated with [^{125}I]-peptide E at 37^{o}C. In both cases incubation yielded [^{125}I]-Leu-E and [^{125}I]-Met-E-Arg^{6}, identified in the manner described for the trypsin digests. The pH optimum for conversion was pH 5.7, which is the internal pH of the chromaffin granules. Significant inhibition of conversion occurred in the presence of 1 mM PCMB, this inhibitory action was prevented by the presence of 1 mM dithiothreitol. The enkephalin producing activity was enhanced by the addition of calcium to the incubation mixture. Subcellular localization indicated that the enzyme activities are not lysosomal in origin; acid phosphatase and catecholamine profiles are completely separated, the enzyme activity profile is coincident with that of the catecholamines. The soluble lysate was further purified by fractionation with ammonium sulfate, the 40 - 100% saturation fraction was resuspended and applied to a Sephadex G-200 column. Enkephalin producing activity was found in the fractions immediately following the void volume. The presence of enkephalin converting enzymes with the precursors and products in the chromaffin granules could be important in the elucidation of the biosynthetic pathway of the enkephalins. This work was supported in part by PHS grants DA02013, MH17785 and MH29591; CMT was supported by MSTP grant GM07038.

130.2 β-ENDORPHIN AND α-N-ACETYL β-ENDORPHIN IN BLOOD: PEPTIDE MARKERS FOR INVESTIGATING THE CONTROL OF RELEASE OF ANTERIOR OR INTERMEDIATE LOBE PITUITARY β-ENDORPHIN IN VIVO. Christopher J. Evans*, Eckard Weber*, Elizabeth Erdelyi*, Robin Lorenz* and Jack D. Barchas. Nancy Pritzker Laboratory of Behavioral Neurochemistry, Stanford Medical Center, Stanford, CA 94305.

The analysis of β-endorphin-like immunoreactivity in blood is of considerable interest since it represents the material actually released by the pituitary gland in vivo. In this study we have characterized β-endorphin in rat trunk blood using a number of region-specific radioimmunoassays interfaced with gel filtration column chromatography. Previously we raised antibodies specific for α-N-acetylated β-endorphin, an endogenous variant which has no opiate-like properties. This antibody in conjunction with antibodies specific for the C-terminus and middle region of β-endorphin have enabled us to immunologically characterize endogenous β-endorphin-like peptides from pituitary and brain. Our results demonstrate that greater than 95 percent of the β-endorphin immunoreactivity in rat pituitary intermediate lobe is α-N-acetylated at the N-terminal tyrosine and shorter than β-endorphin(1-31). Conversely, in the anterior lobe of rat pituitary no acetylated β-endorphin was detected by immunohistoflurescence and the majority of the β-endorphin-like immunoreactivity corresponded to β-endorphin(1-31) and β-lipotropin. When we analyzed blood from normal rats, we found high concentrations of immunoreactive material crossreacting in the acetyl β-endorphin RIA (1.40 ± 0.048 pmoles/ml). However, the middle region and C-terminal directed antibodies detected less material (0.630 ± 0.027 pmoles/ml and 0.120 ± 0.013 pmoles/ml respectively). The acetyl β-endorphin-like immunoreactivity eluted in three peaks from the gel filtration column--the major peak being slightly larger than met-enkephalin sized peptides.

The immunoreactive material recognized by the middle region and C-terminal antibodies eluted between 2 and 3.5 k daltons. No β-lipotropin sized immunoreactive material was detected. In order to demonstrate that the only source of blood endorphin was the pituitary, we assayed blood from hypophysectomized animals--no endorphin-like immunoreactivity was detected. The effects of adrenalectomy were dramatic--immunoreactive material recognized by the C-terminal antibody was elevated nearly 5-fold, an increase which was almost entirely accounted for by the appearance of β-lipotropin in the blood. However, no increase in the concentration of acetylated β-endorphin was observed in adrenalectomized animals suggesting the adrenal feedback that controls the release of anterior lobe pro-opiocortin fragments does not influence the release of the pro-opiocortin fragments of intermediate lobe origin.

130.3 PURIFIED BRAIN ENDOPEPTIDASE PROCESSES ENKEPHALIN PRECURSORS. M. Knight and C.A. Tamminga. Experimental Therapeutics Branch, Natl. Inst. Neurological and Communicative Disorders and Stroke, NIH, Bethesda, Md. 20205.

A membrane-associated endopeptidase has been purified from washed rat brain membranes. The enzyme cleaves small peptides at the peptide bond following methionine and leucine residues. The action of the endopeptidase on endogenous enkephalin precursors of the striatum was studied. An acetic acid striatal extract was gel filtered and enkephalin and enkephalin precursor peptides were measured in the column fractions. The endopeptidase produced as much enkephalin immunoreactivity from the precursor peptides predigested with trypsin as carboxypeptidase B. Endopeptidase alone produced a small increase in enkephalin immunoreactivity. The increase in immunoreactivity was identified by high performance liquid chromatography to be methionine and leucine enkephalin. It was thus shown that a brain enzyme highly concentrated in the striatum can process striatal enkephalin-containing peptides to free enkephalin. Additionally, the endopeptidase produced enkephalin from a proenkephalin peptide, Enk Arg^{6}, by removing the arginine. These studies show that the biosynthesis of enkephalin from the prohormone can proceed with the action of only two peptidases. Initially a trypsin-like peptidase could act on the pre-proenkephalin polypeptide to form small peptide intermediates with amino terminal enkephalin sequences. Then, these peptide intermediates can be processed to free enkephalins in a final processing step requiring this endopeptidase.

130.4 AN ENKEPHALIN-GENERATING ENZYME IN BOVINE ADRENAL CHROMAFFIN GRANULES. I. Lindberg*, H.-Y.T. Yang and E. Costa. (SPON: F. Karoum). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

The adrenal medulla contains large quantities of high molecular weight forms of enkephalin; some of these peptides may represent precursors to met- and leu-enkephalin. We have previously shown that adrenal medullary chromaffin granules contain a trypsin-like enzyme which is capable of generating met-enkephalin from endogenous high molecular weight precursor(s). Using a soluble protein fraction prepared from lysed chromaffin granules, we have partially purified this enzymatic activity by affinity chromatography on soybean trypsin inhibitor coupled to Sepharose. Approximately 0.2% of the lysate protein applied to the affinity column was retained after extensive washing of the column with buffer. This protein was eluted with 0.25 M acetic acid and, following neutralization and concentration, used as the enzyme source; material which was not retained by the affinity column was concentrated, heated, and used as the substrate source. It was shown that the partially purified enzyme retains the ability to generate met-enkephalin as well as other low molecular weight enkephalin-immunoreactive peptides from high molecular weight substrate(s); Peptide F could also serve as the substrate for the enzyme preparation to yield low molecular weight enkephalin-immunoreactive peptides. The generation of enkephalin-immunoreactivity was found to be dependent on the length of incubation and the substrate concentration. Production of met-enkephalin-immunoreactivity was not inhibited by sulfhydryl reagents but was inhibited by soybean trypsin inhibitor, trasylol, and diisopropylfluorophosphate, suggesting that the enzyme is a serine protease and not a cathepsin of lysosomal origin. This conclusion is further supported by the finding that the pH optimum of the enzymatic activity was 7.5-8.0. Further purification of this adrenal trypsin-like enzyme is in progress.

(Supported in part by a PRAT Fellowship to I.L.)

130.5 PURIFICATION AND CHARACTERIZATION OF A SPECIFIC ENKEPHALIN SYNTHESIZING CARBOXYPEPTIDASE. L.D. Fricker and S.H. Snyder. Johns Hopkins University, Sch. of Med., Dept. of Neuroscience, Pharmacology and Psychiatry, Baltimore, MD 21205.

A specific carboxypeptidase which converts enkephalin precursors into enkephalin has been purified and characterized. In the adrenal this enzyme, designated "enkephalin convertase", is uniquely localized to the chromaffin granules, which contain enkephalin and precursor peptides. Brain enkephalin convertase shows 10-fold regional variations which correlates with enkephalin distribution. In the pituitary enkephalin convertase is enriched in the anterior lobe.

Enkephalin convertase has been purified to apparent homogeneity from bovine brain, pituitary, and adrenal medulla. The activation and inhibition of enkephalin convertase with divalent cations and enzyme inhibitors is identical for the brain, pituitary, and adrenal enzymes. Enkephalin convertase is activated 10-fold by 1 mM $CoCl_2$ and is significantly inhibited by 1 mM EDTA or 1,10-phenanthroline.

A rapid, sensitive assay has been developed for both enkephalin convertase and the trypsin-like enkephalin processing endopeptidase. The assay for enkephalin convertase makes use of a solubility change incurred when the water soluble Dansyl-Phe-Leu-Arg is converted to the chloroform soluble Dansyl-Phe-Leu, permitting rapid separation of product from substrate with a sensitivity of 10 picomoles. This assay can be used for other peptide processing enzymes.

130.6 EFFECT OF ENKEPHALINS ON BRAIN MEMBRANE PROTEIN PHOSPHORYLATION. J. Patel, G. Gardner, T. O'Donohue*, and P.J. Marangos*. Clinical Psychobiology Branch, National Institute of Mental Health, 9000 Rockville Pike, Bethesda, MD 20205.

Protein phosphorylation is envisaged to play a pivotal role in cellular functions. In the neuron protein phosphorylation is involved in the regulation various aspects of synaptic transmission. There is evidence that phosphoproteins are involved in regulation of neurotransmitter synthesis, release and its postsynaptic efficacy. In the present study we have examined the possible involvement of protein phosphorylation in the process by which opoid neuropeptides alter synaptic events. The effect of opioid neuropeptides on endogenous protein phosphorylation were studied in vitro using a crude synaptic membrane preparation. The membranes were incubated with ^{32}P-ATP at 30° for 30 seconds in the presence of various peptides and the phosphorylation was terminated with SDS. The phosphoproteins were separated by polyacrylamide gel electrophoresis and autoradiography performed to identify them. Incubation of synaptic membranes with met- and leu-enkephalins resulted in the decrease of phosphorylation of a specific protein of molecular weight 50,000. The half maximal decrease was obtainable at concentrations of 1.5 μm. The inhibitory potency of met- and leu-enkephalin were similar but greater than those obtained with morphine or etorpine. β-endorphin was found to have no effect on protein phosphorylation in our system over the concentration range tested.

None of the several other nonopioid neuropeptides tested were found to affect the phosphorylation of the 50K protein. The 50K protein represented a major phosphoprotein in a well washed membrane preparation. This protein was found to be present in all the brain areas investigated with enrichment in hippocampus and olfactory bulb. Work is presently in progress to determine whether the observed phosphorylation inhibition is mediated by the opiate receptor. Our results suggest that it is possible that modulation of protein phosphorylation by opoids may represent an underlying mechanism by which these agents exert their effect.

130.7 REGULATION OF MET- AND LEU-ENKEPHALIN LEVEL IN RAT ANTERIOR PITUITARY BY SEX HORMONES. K. Yoshikawa* and J.S. Hong. Lab. of Behavioral and Neurological Toxicology, NIEHS, Research Triangle Park, NC 27709

The pituitary contains a variety of opioid peptides - e.g., β-endorphin, met-enkephalin (ME), leu-enkephalin (LE), dynorphin, etc.; however, very little is known concerning the regulation of the pituitary enkephalin system. Recently, we observed a marked sex-related difference in ME-immunoreactivity (ME-IR) in rat pituitary. The purpose of this study was to investigate the mechanism underlying the sex-related difference in the pituitary enkephalin system.

An ontogenic study of rat pituitary ME-IR revealed that the sex-related difference in the level of ME-IR became apparent at 70 days of age. At 180 days of age, pituitary ME-IR of male rats was twice as much as that of female rats. When male rats were castrated at one day of age and sacrificed at 120 days of age, pituitary ME-IR of the castrated rats was about 40% of sham-castrated animals. On the other hand, there was a 50% increase of the pituitary ME-IR when female rats were ovariectomized at 55 days and sacrificed at 120 days of age. The increase of ME-IR by ovariectomy was, however, completely eliminated by the concurrent administration of estrogen.

When the pituitary was divided into anterior lobe and posterior lobe (together with the pars intermedia), the sex-related difference of ME-IR was evident only in the anterior but not in the posterior lobe. LE-immunoreactivity (LE-IR) also showed a distinct sex-related difference only in anterior lobes. Chemical characterization using reverse-phase HPLC showed that the sex-related difference in ME-IR and LE-IR reflected an actual change of authentic ME and LE in the anterior lobe. Castration or estrogen administration caused a marked decrease in both ME-IR and LE-IR in the anterior lobe of male rats. The diminished level of enkephalins induced by castration was partially restored by dihydrotestosterone administration. On the other hand, ovariectomy or dihydrotestosterone administration resulted in a significant increase in ME-IR and LE-IR in the anterior lobe of female rats. These results suggest that ME and LE in the anterior pituitary may be regulated through a common mechanism by gonadal steroid hormones.

We also found that repeated injections of haloperidol or LiCl caused a significant decrease of ME-IR and LE-IR in the anterior lobe. Since estrogen exerts an anti-dopaminergic effect in the pituitary, it is likely that the suppressive effect of estrogen on the enkephalin system in the anterior pituitary is attributable, at least in part, to this anti-dopaminergic trait.

130.8 EFFECT OF AMINOPEPTIDASE INHIBITORS, BESTATIN AND PUROMYCIN ON ENKEPHALIN ACTIVITY AND METABOLISM IN THE GUINEA PIG ILEUM. Laurane E. Geary and Marlene L. Cohen*. Lilly Research Labs., Eli Lilly and Company, Indianapolis, IN 46285.

Aminopeptidases are the primary enzymes involved in enkephalin degradation in the guinea pig ileum. Enkephalinases, although present in the ileum, appear less important to the degradation of enkephalins since thiorphan, a potent enkephalinase inhibitor, did not potentiate enkephalin responses or prevent enkephalin degradation in this tissue. (Geary et al, J. Pharmacol. Exp. Ther. 221:104, 1982). In the present study, puromycin, an arylamidase inhibitor, and bestatin, a leucine aminopeptidase inhibitor, were examined for their ability to (1) potentiate Met- and Leu-enkephalin mediated inhibition of the field stimulated (40 V., 0.7 msec. duration, 0.1 Hz) contracted ileum and longitudinal muscle, and (2) alter the formation of [^{3}H]Tyr and [^{3}H]Tyr-Gly-Gly from [^{3}H]Met- and Leu-enkephalin. [^{3}H]Met- and Leu-enkephalin and metabolites were measured by HPLC and liquid scintillation spectroscopy.

Although puromycin has been reported to block enkephalin degradation in homogenates from the guinea pig ileum, puromycin (10^{-4}M) had no effect on the potency, duration or metabolism of Met- or Leu-enkephalin in the intact ileum. In the longitudinal muscle, puromycin (10^{-4}M) enhanced the duration of enkephalin-induced inhibition without altering the maximal response or the degradation of [^{3}H]enkephalin. Therefore, the effect of puromycin on the duration of enkephalin activity in the longitudinal muscle is not related to alterations in enkephalin metabolism.

In contrast to puromycin, bestatin (10^{-7}M - 10^{-5}M) produced a dose-dependent increase the potency and duration of Met- and Leu-enkephalin-induced inhibition in the intact ileum and longitudinal muscle. These actions of bestatin (10^{-5}M) were associated with a reduction in [^{3}H]Tyr (80 °/o inhibition) and an increase in [^{3}H] enkephalin (60 °/o increase) in the intact ileum. The reduction in [^{3}H]Tyr by bestatin (10^{-5}M) was also accompanied by a significant increase in [^{3}H]Tyr-Gly-Gly measured in the ileum. Total radioactivity found as [^{3}H]Tyr-Gly-Gly increased from 5 °/o (control) to 10 °/o (bestatin). Thus, bestatin, but not puromycin, potentiated the enkephalin-mediated inhibition of the twitch response and decreased enkephalin degradation in the ileum. This indicates that leucine aminopeptidases, but not arylamidases, are important for enkephalin inactivation in this tissue.

130.9 PLASMA, PITUITARY, MIDBRAIN AND HYPOTHALAMIC BETA-ENDORPHIN IMMUNOREACTIVITY IN PREGNANT RATS. C. Cahill*, H. Akil, (SPON: R. Davis). Mental Health Research Institute, University of Michigan, 48109.

Beta-endorphin (β-END) is thought to be involved in the mediation of pain perception in animals and man. Several groups have shown that plasma β-END-like immunoreactivity is elevated during pregnancy and labor in humans. Our studies of plasma β-END in pregnant women demonstrate that immunoreactivity rises significantly during the third trimester of pregnancy and during labor, and drops precipitously post partum to non-pregnant levels. However, we have been unable to demonstrate a relationship between self-reported pain perception during labor and plasma β-END levels. It is therefore critical to further characterize the immunoreactive plasma β-END in pregnancy and ascertain its size and opiate activity. Our biochemical studies demonstrate that the majority of immunoreactive material is indeed β-END-sized. However some of it may be N-acetylated, raising questions as to the source of the material (anterior pituitary, intermediate lobe or fetal), or atypical processing.

Therefore, in this study, we will address and report on the following related issues: a) biochemical characterization of plasma β-END in pregnant vs. non-pregnant rats in order to determine molecular size and acetylation; b) comparison of the biochemical characteristics of plasma β-END in pregnant humans to rats; c) biochemical characterization of β-END in anterior, posterior/intermediate lobe pituitary, hypothalamus and midbrain in pregnant rats vs. non-pregnant controls in order to determine the possible source of the plasma β-END material as well as to explore the relationship between plasma and central changes.

130.10 PEPTIDE F (PRO-ENKEPHALIN PRECURSOR FRAGMENT): ADRENAL MEDULLARY AND CENTRAL NERVOUS SYSTEM (CNS) DISTRIBUTION. N. Alessi, L. Taylor[1], H. Akil (SPON: G. Goldstein). Mental Health Research Inst., Univ.[1] of Mich., Ann Arbor, MI 48109; Lafayette Clinic, Detroit, MI.

The enkephalin precursor has been fully sequenced in both humans and bovine adrenal medulla. (Noda et al., Nature, 1982; Gubler et al., Nature, 1982; Combs et al., Nature, 1982). The precursor, pre-proenkephalin has been shown to contain not only Met^5- and Leu^5-enkephalin, but a variety of other polypeptides fragments, including Peptide A through I and BAM-22P. The metabolic pathways and physiological functioning of these fragments remain unknown to date.

Utilizing a nine amino acid (Asp-Glu-Leu-Tyr-Pro-Leu-Val-Glu) non-enkephalin containing fragment of the pro-enkephalin molecule a radioimmunoassay (RIA) was developed. The antiserum was used at a dilution of 1:1500, and did not show cross-reactivity with Met^5-enkephalin, Leu^5-enkephalin, ACTH-(1-27), dynorphin-(1-17), alpha-MSH, or beta-endorphin. The resulting IC_{50} was approximately 25 Fmol. The studies of bovine adrenomedullary chromaffin granule preparations, rat adrenomedullary preparations, and rat brain regions using this RIA have demonstrated that this fragment is present and apparently conserved across species. The highest concentrations of immunoreactivity in rats was found in the adrenal medulla, striatum, cortex, and medulla-pons. Molecular sizing using a Biogel P-2 column, with 2N acetic acid suggest that the fragment measured in these preparations is small (molecular weight less than 1500). Studies to further characterize this peptide using a combination of molecular sizing and a coupled HPLC/RIA procedure are ongoing.

In rats, footshock stress, which produces naloxone-reversible analgesia leads to a significant ($p < .01$) decrease of the immunoreactive material in the adrenal medulla. The value of the "F" fragment in the adrenal medulla's of control animals was 185 ± 27.30 fmol/mg (wet weight) and the value after actue stress was 102 ± 20.60 fmol/mg (wet weight). Chronic daily stress for two weeks which leads to "tolerance" of stress induced analgesia, results in an inability of the adrenal to alter F levels upon subsequent stress. These finidngs are consistent with the alterations found in enkephalin levels under similar condition (Viveros, personal communication).

The existence of the F-like immunoreactivity in a small molecular weight form, and its alteration by environmental manipulation, suggest it may play a unique physiological role.

130.11 THE SIGNAL PEPTIDE OF PRO-OPIOMELANOCORTIN: VALIDATION OF ANTISERUM AND PITUITARY CHANGES WITH STESS H. Akil, S.J. Watson, H. Shiomi*, R. Thompson*, and D. Coy[1]. Mental Health Res. Inst., Univ. of Mich., Ann Arbor, MI 48109; [1]Tulane University, New Orleans, LA 70196.

The precursor of ACTH/beta-endorphin (β-END) and α MSH, also known as POMC, has a signal sequence at its N-terminus. According to the signal hypothesis this sequence is critical for coding that the protein is destined for packaging or membrane incorporation. Signal sequences are thought to be cleaved off extremely rapidly upon translation to protein. We therefore reasoned that the extremely short half life of this region may be useful as an indicator of ongoing biosynthesis of POMC. In sum, the ratio of pre-pro hormone (POMC + signal) to pro-hormone may be an index of the dynamic state of the system.

Part of the POMC signal region was synthesized, an antibody raised, and a high titer radioimmunoassay calibrated. Since it is not possible to study cross-reactivity with other signal peptides, we carried out the following validation scheme: RNA from ovine pituitary was prepared and translated using a rabbit reticulocyte cell free translation system, labelling proteins with ^{35}S methionine. Such a translation system produces pre-pro hormones, with signal sequence attached.

The protein products were extracted and applied to two antibody affinity columns: A β-END-antibody column which is known to capture POMC, and a signal peptide antibody column. A portion of the eluant from each of the columns was then applied on the other column, to check to see if they were binding the same molecule. Eluants from the original columns and the cross runs were then studied on SDS disc gel electrophoresis. All eluants showed a single peak of about 31,000 molecular weight, corresponding to the pre-pro hormone form of POMC without glycosylation. This peak could be immunoprecipitated by β-END, ACTH or signal peptide antisera. We therefore concluded that we had translated fully the POMC pre-pro hormone with its leader sequence. Furthermore, our signal antiserum only cross-reacted with that protein and no other in pituitary.

We then studied the effect of acute footshock stress on signal-immunoreactivity and β-END immunoreactivity in anterior pituitary. Thirty minutes of stress led to a significant depletion of β-END in rat anterior lobe. During the next two hours, -END recovered to normal. Meanwhile, signal immunoreactivity exhibited a four-fold increase suggesting active novel synthesis of POMC. Thus, we have validated a POMC signal RIA, and have shown that its measurement may be a useful tool for studying ongoing novel synthesis of POMC.

130.12 OPIATE PEPTIDE PRECURSORS IN THE ADRENAL MEDULLA. Dane Liston and Jean Rossier. Laboratoire de Physiologie Nerveuse, C.N.R.S., 91190 Gif-sur-Yvette, France.

The discovery of enkephalin-containing proteins (ECP) in the adrenal medulla has led to the use of this tissue as a model to study the processing of enkephalin precursors. Experiments in our laboratory are focusing on the purification and characterization of ECPs with a molecular weight greater than 10,000 daltons derived from chromaffin granules. Bovine adrenal glands were obtained fresh from the slaughterhouse and chromaffin granules were isolated by the method of Smith and Winkler. The granules were lysed in hypotonic medium and the resulting supernatant was subjected to biochemical analysis. Enkephalin immunoreactivity (Enk-IR) was determined following digestion of proteins with trypsin and carboxypeptidase B using antisera directed against either methionine-enkephalin (Met-enk) or leucine-enkephalin (Leu-enk). Exclusion chromatography of the chromaffin granule lysate revealed several peaks of Enk-IR, the largest with an apparent molecular weight of 25-30,000 daltons. The fractions containing this peak were applied to an anion-exchange column and eluted with a linear pH gradient from pH 7 to 4. A peak of Enk-IR eluted in the pH range of 5.1 to 4.4. Isoelectric focusing of these fractions confirmed the presence of an acidic ECP, yielding a band of activity with an isoelectric pH of 4.8. HPLC analysis of a tryptic digest of this protein demonstrated Met-enk-lysine^6 and Met-enk-arginine^6 in a ratio of approximately 3:1. Leu-enk was not found in this protein. Furthermore, examination of acetic acid extracts of bovine adrenal medulla has failed to reveal a Leu-enk-containing protein with a molecular weight greater than 10,000 daltons. Recently several groups have reported the primary sequence of adrenal proenkephalin determined by cloning and sequence analysis of cDNA. The complete sequence contains Leu-enk and several copies of Met-enk. The absence of Leu-enk in the protein we have analyzed suggests that cleavage of the Leu-enk-containing region from the rest of the protein is an early event in the processing of the precursor. In addition, there are two potential sites at which carbohydrates may be conjugated to the protein backbone. In view of the large number of basic amino acid residues present in the protein, it seems likely that the acidic nature of the precursor we have purified arises from a carbohydrate side chain. Such post-translational modification may play an important role in the processing of ECPs into smaller opiate peptides. (Supported by The Esther A. and Joseph Klingenstein Fund.)

130.13 QUANTIFICATION OF OPIOID PEPTIDES IN TRIGEMINAL SENSORY TERMINI, F.S. Tanzer* and D.M. Desiderio. Stout Neuroscience Mass Spectrometry Laboratory, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163.

The dental pulp is a major trigeminal sensory terminus. Pain is considered to be the major sensory response of teeth (trigeminal sensory termini) to stimuli. This tissue contains molecular factors which participates in nociception. This report of dental pulp research describes a novel analytic method for the study of minute tissue samples at the molecular level.

Anesthetized dogs are exsanguinated through a femoral artery, dental pulps removed, immediately placed in liquid nitrogen, and stored at -74°C until use. Weighed pulps are treated with 1 N acetic acid, and internal standard ^{2}alanine-leucine enkephalin, [^{2}ala-LE] added, centrifuged, the supernatant lyophized, reconstituted with trifluoroacetic acid, placed on a Waters ODS C18 sep-pak, eluted with an acetonitrile: triethylamine formate buffer, and lyophilized. The sample is injected on reverse phase high pressure liquid chromatography (RP-HPLC) and peaks collected. By means of synthetic standards, peaks corresponding to known opioids were identified. Enzymatic studies using trypsin, α-chymotrypsin and carboxypeptidase B verified the peptide nature of the isolated supernatant material. The endogenous opioid fractions are then quantified with a Finnigan MAT 731 field desorption mass spectrometer (FD-MS) instrument using peak switching between selected $(M+H)^{+}$ ions of the opioids and ^{2}ala-LE. RP-HPLC and FD-MS provide a powerful method to qualitatively and quantitatively measure biologically active molecules at the part per billion level in dental pulp. Quantitative results indicate that dog dental pulp contains neuropeptides considered factors in the endogenous analgesia system.

This study was supported by Noel Foundation and NIH GM NS 26666.

130.14 REGIONAL DISTRIBUTION OF ENKEPHALIN AND DYNORPHIN IMMUNOREACTIVITY WITHIN THE RAT HIPPOCAMPUS. C. Chavkin, J. McGinty, W.J. Shoemaker, and F.E. Bloom. The Salk Institute, La Jolla, CA 92037. A. Bayon. Instituto de Investigaciones Biomedicas, U.N.A.M., Apartado Postal 70228 Mexico 20, D.F.

In spite of early evidence showing marked effects of iontophoretically applied enkephalin on hippocampal pyramidal cells, initial histochemical studies revealed only scattered enkephalin immunoreactive (enk-ir) fibers in this structure. More recent work, with improved techniques, has shown a wider distribution of enk-ir containing elements in the hippocampus (HC). Additionally, recent immunohistochemical work by one of us (J. McGinty et al. this vol.) showed intense dynorphin-IR staining in the dentate-mossy fiber pathway innervating the CA3 region of the HC. A knowledge of the distribution and precise chemical nature of the endogenous opioids in the HC is a requisite for understanding their physiological roles and relationships. We have developed a rapid dissection procedure that allows us to reproducibly divide blunt dissected HC into four regions: dentate-CA4; CA2-3; CA-1; and subiculum. Additionally, because it contains an enk-IR projection to HC, entorhinal cortex was dissected from the ventrodorsal surface of the brains, and included in the study. Pools of 10-20 fragments from each region were incubated for 30 min at 90° in 10 vol 1M acetic acid, homogenized, then centrifuged 30 min at 40,000 xg. Supernatants were analyzed for dynorphin-IR, leu-enk-IR, and β-endorphin-IR contents. The extracts were also resolved in both HPLC gel-permeation columns and C18 reverse phase HPLC columns. Enkephalin levels in the CA2-3 region are more than 5-fold greater than in the CA1 and subiculum. The dentate-CA4 and entorhinal cortex regions contain about 50% of the enk-IR found in CA2-3. Preliminary results indicate that the β-endorphin-IR detected by RIA in the hippocampus resides in substances of molecular weight less than β-endorphin. The leu-enkephalin RIA used could also detect other endogenous opioids sharing its sequence: met enkephalin, α-neo-endorphin, dynorphin and possibly others. In fact, immunohistochemical results demonstrate that the enkephalin-IR in CA2-3 and dentate-CA4 is likely to be largely due to dynorphin, and in agreement with above, analysis of the dynorphin-IR content demonstrated that its concentration ranking was CA2-3 > dentate-CA4 >> CA1 = entorhinal cortex = subiculum. Resolution of the opioid peptides extracted from each region on gel-permeation and reverse phase HPLC allows the molecular characterization and quantitation of dynorphin and enkephalin in hippocampus. (This study was supported by grants NIDA 01785 and NIAAA 07273.)

130.15 OPIOID PEPTIDES AND VASOPRESSIN: DISTRIBUTION IN SUBCELLULAR ORGANELLES OF RAT PITUITARY AND HYPOTHALAMUS. C.J. Molineaux*, T. Alhadi and B.M. Cox. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Md. 20814

The potent opioid peptide, dynorphin (DYN), has been shown to be associated with the arginine-vasopressin (AVP) containing magnocellular neurons of the hypothalamo-hypophysial tract (Watson, et al., Science 216:85, 1982). However, comparison of the distributions of DYN-(DYN-LI) and AVP-(AVP-LI) like immunoreactivities in discrete nuclei of the hypothalamus suggests that DYN is also stored in structures that contain little or no AVP (Molineaux, et al., in prep.). In contrast, another opioid peptide, α-neo-endorphin (α-neo-END), has been reported to have a similar distribution to DYN in hypothalamus (Weber, et al., Biochem. Biophys. Res. Com. 103:951, 1981). In this communication, we have compared the subcellular distributions of AVP-LI, DYN-LI and α-neo-END-LI in rat hypothalamus, and neurointermediate (NIL) and anterior (AL) lobes of pituitary.

In all tissues, most of the peptide immunoreactivity was found in a particulate fraction, which was further fractionated by centrifugation into a sucrose density gradient (0.6-1.6 M). AVP-LI sedimented to a similar position in both NIL and hypothalamus extracts. In NIL samples, the distribution of DYN-LI and α-neo-END-LI was similar to that of AVP-LI, with respect to both rate of sedimentation and final equilibrium position, suggesting storage of these peptides in organelles of similar size and density. In contrast, in hypothalamus, most of the DYN-LI and α-neo-END-LI were found in a less dense region of the gradient. Only a small fraction of the hypothalamic DYN-LI and α-neo-END-LI co-sedimented with AVP-LI. The results suggest that the bulk of the dynorphin and α-neo-endorphin in the hypothalamic samples does not originate from the magnocellular neurons.

In anterior lobe of pituitary, DYN-LI and α-neo-END-LI also co-sedimented. Two components, each containing both immunoreactivities, were observed; one in the same position as the granules containing DYN-LI from the NIL, the other in an even more dense region of the gradient. Our results emphasize the close association of DYN and α-neo-END in three different tissues.

130.16 **IN VITRO CENTRAL β-ENDORPHIN METABOLISM IN SCHIZOPHRENIA.** **Hans Schoemaker*, Henry I. Yamamura and Thomas P. Davis. Dept. of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724.**

The in vitro metabolism of β-endorphin (βE, βLPH 61-91) results in the formation of among others αE (βE 1-16), γE (βE 1-17), and their non-opiate des-tyrosine^{1}-analogs. On basis of the amphetamine- and neuroleptic-like activity of the α- and γ-type endorphins, respectively, previous studies have suggested that the endorphins may function in brain homeostasis and a variety of behavioral adaptive processes. This has led to the hypothesis that a dysfunction in βE metabolism, resulting in an altered balance between the α-, β- and γ-type endorphins could underly schizophrenic psychoses. This hypothesis is supported by the therapeutic effects of des-tyr-γ-E and des-enkephalin-γ-E in schizophrenia. In order to address this hypothesis, in vitro βE metabolism by membrane bound enzymes from the post-mortem caudate nucleus of control and schizophrenic subjects was studied. βE (20 μM) was incubated in PBS buffer at 37°C with twice washed membrane preparations of the post mortem caudate nucleus. The samples were previously stored at -80°C for 4-5 years. After 30-120 min incubations, samples were boiled for 10 min and centrifuged for 60 min at 15,000xg. The supernatant was assayed for βE related peptide fragments by means of a selective and quantitative HPLC procedure. Peptides were separated on an Ultrasphere ODS 5μ column using a 16 to 32% curvelinear gradient of acetonitrile against 0.1 M NaH_2PO_4 buffer (pH 2.1) over 50 min using a flowrate of 2 ml/min at a temperature of 40°C. Quantitation was by UV absorbance at 210 nm. βE metabolism was characterized using washed membrane preparations of the caudate nucleus of 3 control and 3 schizophrenic subjects. Within this small sample size, a tendency towards both qualitative and quantitative differences in metabolism were apparent. The most striking difference was the greatly reduced level of the peptide fragment coeluting with βE 6-21 from schizophrenic post mortem caudate nucleus versus age and sex matched controls. Furthermore, quantitative differences in both the α-type and the γ-type endorphins were present. These data await our confirmation in a larger sample size of brain tissues from control and schizophrenic subjects. The question of whether these differences in βE metabolism in schizophrenia are drug-induced or inherent to the disease state, needs to be addressed. The functional relevance of these in vitro findings for in vivo βE biotransformation awaits future research. **Supported in part by USPHS grants MH-27257 and MH-30626 and a RSDA type II to HIY, and a BRSG and PMA grant to TPD.**

131.1 EFFECTS OF SYSTEMICALLY ADMINISTERED DOPAMINE AGONISTS ON GLOBUS PALLIDUS SINGLE CELL ACTIVITY: REFLECTION OF POSTSYNAPTIC D-2 RECEPTOR STIMULATION? D.A. Bergstrom, D.M. Jackson and J.R. Walters. NINCDS, NIH, Bethesda, MD 20205.

Previous studies have demonstrated that systemically administered apomorphine can affect tonically firing cells in the globus pallidus (external pallidum) of locally anesthetized, gallamine-paralyzed and artificially respired rats (1). Doses of 1 μmol/kg of apomorphine or the ergot dopamine agonists, lisuride or pergolide, produce an average increase of 100% in the tonic activity of these neurons; an effect blocked or reversed by haloperidol. d-Amphetamine, but not l-amphetamine, causes similar increases in pallidal cell firing rates (2). The present study explores the selectivity of this effect.

Standard extracellular single unit recording techniques were used to record pallidal activity in gallamine-paralyzed, locally anesthetized and artificially respired rats. To determine whether serotonin receptor stimulation activates pallidal firing rates in a manner comparable to that of dopamine receptor stimulation, the effects of d-lysergic acid diethylamide (LSD) were examined. LSD is a potent serotonin agonist with dopamine agonist properties considerably weaker than those of lisuride and pergolide. A 1 μmol/kg i.v. dose of LSD produced only a 6 ± 16% increase in pallidal activity (n=11). Norepinephrine agonists have been shown to induce considerably smaller changes in pallidal activity than do dopamine agonists (2). Together, these results suggest that dopamine receptor stimuation is more effective than serotonin or norepinephrine receptor stimulation at inducing increases in pallidal activity.

The effects of dopamine agonists with putatively selective actions on specific subcategories of dopamine receptors were also examined. SK&F 38393, a drug which preferentially stimulates D-1, as opposed to D-2, dopamine receptors in the striatum, and 3-PPP, a drug thought to preferentially affect presynaptic dopamine receptors, were administered i.v. SK&F 38393, in doses of 1 and 30 μmol/kg, had no significant rate effect (18 ± 7% increase, n=8 and 1 ± 7% decrease, n=10, respectively) on pallidal activity. 3-PPP (15 μmol/kg) also induced no change in pallidal firing rates (7± 15% increase, n=10) although smaller doses of 3-PPP inhibited the activity of dopamine cells in the substantia nigra pars compacta (ED_{50}=.3±.1 μmol/kg, n=9).

These results suggest that drugs thought to be effective at selectively stimulating D-1 or presynaptic dopamine receptors do not significantly alter pallidal firing rates. The changes which have been observed with apomorphine, lisuride and pergolide appear to be related to their postsynaptic D-2 dopamine receptor stimulating properties.

(1) Bergstrom, D.A. et al. Europ. J. Pharmacol. 78: 245, 1982.
(2) Bergstrom, D.A. and Walters, J.R. J. Neurosci. 1: 292, 1981.

131.2 EVIDENCE FOR A PHYSIOLOGICAL ROLE OF DOPAMINE AS A MODULATOR OF GABA EFFECTS ON SUBSTANTIA NIGRA PARS RETICULATA NEURONS. B.L. Waszczak, E.K. Lee*, C.A. Tamminga and J.R. Walters. NINCDS, NIH, Bethesda, MD, 20205.

Previous studies in this laboratory have demonstrated that iontophoretically-applied dopamine (DA) can consistently and markedly diminish the inhibitory effects of iontophoresed GABA on neurons of the substantia nigra (SN) pars reticulata. It was of interest to determine whether similar modulatory interactions between DA and GABA could occur as a consequence of endogenous, local release of DA from dendrites of neighboring pars compacta DA neurons. These studies were undertaken to assess whether d-amphetamine (AMPH), a drug reported to induce release of DA from dendrites within the SN, could also attenuate responses of reticulata neurons to GABA.

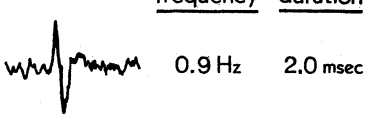
Extracellular, single unit activity of SN pars reticulata neurons was recorded in male rats anesthetized with chloral hydrate. For each cell, repeated 30 sec iontophoretic pulses of GABA (0.001 M in 0.2 M NaCl), separated by 30 sec baseline periods, were delivered at an ejection current sufficient to inhibit reticulata cell firing by at least 50%, but not totally. After establishing a consistent response to GABA, AMPH (1.6 mg/kg) was administered i.v., and responses to GABA were compared with those before AMPH. For 7 of the 15 cells tested (47%), AMPH reduced the number of spikes inhibited by GABA by an amount greater than 10% of the pre-drug baseline firing rate. Of the remaining cells, 6 (40%) exhibited attenuated responses of a somewhat lesser magnitude (between 5 and 10% of their pre-drug baseline rates), while for 2 cells the number of spikes inhibited by GABA was essentially unchanged after AMPH. Although AMPH had variable effects on the baseline firing rates of many cells, these changes seemed to occur independently of the drug's frequent ability to attenuate response to GABA.

Unlike its effects in normal rats, i.v. AMPH (1.6 mg/kg) did not reduce reticulata cell responses to GABA in rats which received treatments to deplete or destroy nigral DA stores. In rats pretreated with reserpine (1.5 mg/kg) and α-methyl-p-tyrosine (250 mg/kg) (n=7), or in rats studied 1-4 weeks after unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigro-neostriatal pathway (n=12), AMPH did not lessen the number of spikes inhibited by GABA. However, in that group of 6-OHDA-lesioned rats studied 3-4 weeks after the lesions, the ability of iontophoretically-applied DA to attenuate reticulata responses to GABA tended to be enhanced relative to that of unlesioned rats (p=.05, n=28). This finding suggests that the DA receptors involved in the modulatory interaction may ultimately become supersensitive in a DA deficient SN.

In summary, the ability of AMPH to attenuate reticulata cell responses to GABA, observed only in rats with intact nigral DA systems, provides evidence for a physiological role of DA, presumably released from dendrites, as a modulator of GABA effects on SN pars reticulata neurons projecting to pre-motor nuclei outside the basal ganglia.

131.3 EFFECT OF DOPAMINE AGONISTS ON THE ACTIVITY OF SUBSTANTIA NIGRA NEURONS IN OCULO, S.M. Wuerthele, B.J. Hoffer* and L. Olson*†. University of Colorado Health Sciences Center, Denver, CO 80262, and †Karolinska Institute, Stockholm, Sweden.

Ventral mesencephalic tissue containing substantia nigra has been successfully transplanted from 17-day-old rat fetuses into the anterior chamber of the eye of adult rat hosts. These tissues produce dopamine (DA)-containing fibers which are capable of uptake of, and when electrically stimulated, release of exogenous dopamine in vitro. Furthermore, electrically-stimulated release of dopamine from these tissues is inhibited by the DA agonist apomorphine, and increased by the DA antagonist pimozide (Olson and Seiger; Z. Zellforsch 135: 175, 1972; Seiger, Olson and Farnebo, Cell Tiss. Res. 165: 157, 1976). Since these transplants lack postsynaptic central neuronal targets, such data suggests that dopamine release from substantia nigra neurons is influenced by presynaptic dopamine receptors. We have carried out extracellular recordings from nigral transplants in oculo using NaCl-filled micropipettes. We find spontaneously active neurons with action potential waveforms (1.5-2.5 msec duration; distinct break between initial segment and somatodendritic component) and firing rates (0.5-8.0 Hz) similar to that previously reported for substantia nigra transplants in the lateral ventricle (Wuerthele et al., Exp. Brain Res. 44: 1, 1981) and for substantia nigra in situ.



Furthermore, local application of dopamine agonists inhibits the activity of such neurons in a dose-dependent manner. These data suggest that, in addition to regulating the release of dopamine, presynaptic receptors may also influence the electrical activity of dopamine-containing neurons of the substantia nigra.

(Supported by USPHS Grants NS-09199 and NS-06659, and by Swedish MRC Grant 14X-03185.)

131.4 MESOCORTICAL DOPAMINE NEURONS. I. ELECTROPHYSIOLOGICAL AND BIOCHEMICAL EVIDENCE FOR THE ABSENCE OF AUTORECEPTORS IN A SUBPOPULATION. M. J. Bannon, L. A. Chiodo, R. H. Roth, and B. S. Bunney. Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510

The large increase in striatal and olfactory tubercle DA levels elicited by γ-butyrolactone (GBL) injection can be prevented by apomorphine acting at DA nerve terminal autoreceptors. In contrast, the effects of GBL in the prefrontal cortex were unaffected by apomorphine (i.e., the mesocortical DA neurons innervating the prefrontal cortex lack terminal autoreceptors; Bannon et al., Nature 296: 444, 1982). In the present study we used single-unit electrophysiological and biochemical techniques to examine for the presence or absence of both nerve terminal and somatic/dendritic autoreceptors on mesocortical DA neurons projecting to the prefrontal, cingulate and entorhinal cortices.

By testing the ability of apomorphine to reverse the GBL-induced elevation of DA levels within these regions it was determined that the DA neurons innervating the cingulate and prefrontal cortices lack terminal autoreceptors while those DA neurons projecting to the entorhinal cortex possess them. Using antidromic activation to identify the projection area of the DA cells studied, and both the iontophoretic application of DA and the systemic application of apomorphine to test for the presence of autoreceptors, it was observed that DA neurons innervating the prefrontal and cingulate cortices were also devoid of somatic/dendritic autoreceptors while those cells which terminated within the entorhinal cortex were shown to possess these receptors.

In agreement with previous data (Bannon et al., Brain Res. 218: 376, 1981) the DA turnover in the prefrontal cortex (measured as the decline of DA 30 minutes after α-methylparatyrosine) was significantly faster than striatal DA turnover (48% vs. 21% depletion, respectively). The DA turnover in the cingulate but not the entorhinal cortex was significantly faster than the striatal turnover. A strong correlation was also present between the turnover rates within the various cortical regions and the neuronal discharge rates observed in antidromically identified cells. That is, mesocortical DA neurons which innervated the prefrontal and cingulate cortices had significantly higher firing rates and displayed a higher incidence of burst discharges than did those cells projecting to the entorhinal cortex.

(This work was supported by USPHS grants MH-25642, MH-14276, MH-14092, NIH fellowship NS-07136 and the State of Connecticut.)

131.5 MESOCORTICAL DOPAMINE NEURONS. II. PRESENCE OR ABSENCE OF AUTORECEPTORS PREDICTS RESPONSIVENESS TO DOPAMINE AGONISTS AND ANTAGONISTS. R. H. Roth, M. J. Bannon and M. E. Wolf*. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

The mesocortical dopamine (DA) neurons projecting to the prefrontal and cingulate cortex were found by means of electrophysiological and biochemical techniques to be devoid of DA autoreceptors, while those projecting to the entorhinal cortex possessed functional DA autoreceptors (see preceding abstract). The administration of autoreceptor-selective doses of apomorphine or the putative DA autoreceptor agonist, 3-PPP, caused significant (as large as 35%) reductions in the levels of the DA metabolite homovanillic acid (HVA) in midbrain DA neurons projecting to the striatum, olfactory tubercle and entorhinal cortex but was without effect on HVA levels in the mesocortical DA neurons innervating the cingulate and prefrontal cortices. Similarly, systemic administration of the DA antagonist haloperidol produced large increases (in the range of 100-250%) in the accumulation of the DA metabolite dihydroxyphenylacetic acid (DOPAC) in the nigrostriatal and mesolimbic DA projections and in the mesocortical DA neurons innervating the entorhinal cortex, but elicited only modest increases (30-50%) in DOPAC levels in the mesocortical DA neurons (those lacking autoreceptors) projecting to the prefrontal and cingulate cortices. These pharmacological data are consistent with the hypothesis that the absence of autoreceptors may explain the unique responsiveness of some mesocortical DA systems, including: the diminished effect of dopaminergic drugs, the lack of tolerance development seen in these systems following chronic antipsychotic drug administration, and the enhanced responsiveness of these systems to mild stress. (Supported in part by USPHS Grant #MH-14092 and the State of Connecticut.)

131.6 NALOXONE POTENTIATION OF APOMORPHINE-INDUCED STEREOTYPIC CLIMBING IN MICE: INFLUENCE OF DRUG PRETREATMENTS THAT ALTER LEVELS OF BRAIN CATECHOLAMINES. Raymond M. Quock and Alan S. Bloom. Div. Pharmacology, Marquette University School of Dentistry, Milwaukee, WI 53233 and Dept. Pharmacology & Toxicology, The Medical College of Wisconsin, Milwaukee, WI 53226.

Previous investigations in our laboratory have consistently demonstrated that pretreatment with narcotic antagonist drugs potentiates the effects of dopaminergic agents in a number of paradigms (Quock, *Life Sciences* 20:2005, 1977; Quock and Welsh, *J. Pharm. Pharmacol.* 33:111, 1981; Quock and Lucas, *Life Sciences* 28:1421, 1981; Namba *et al.*, *Life Sciences* 28:1629, 1981). We have speculated that such potentiation of dopaminergic drug effect might be attributed to blockade of opiate receptors situated on dopaminergic nerve terminals and that are inhibitory to dopaminergic neuronal function. We decided to test this hypothesis by pharmacologically manipulating central dopamine (DA) and norepinephrine (NE) levels and assessing such influence upon naloxone (NX) potentiation of the stereotypic climbing response induced in mice by apomorphine (APO). α-Methyl-*p*-tyrosine (αMT) [150 mg/kg as the methylester HCl, ip, 2 hr pretreatment time] reduced brain DA by ∿50% and brain NE by ∿40%; such pretreatment failed to suppress APO climbing but did completely reverse NX potentiation of APO stereotypy. Sodium diethyldithiocarbamate [400 mg/kg, ip, 2 hr] reduced brain DA by ∿30% and brain NE by ∿30%; as with αMT, such pretreatment failed to suppress APO climbing but did completely reverse NX potentiation of APO stereotypy. These experiments tend to suggest that endogenous catecholamine levels are required for manifestation of NX potentiation of APO climbing. This was also supported by an additional experiment in which bilateral pretreatment with 6-hydroxydopamine HBr [5 µg, icv, 48 hr] failed to suppress APO climbing but also completely reversed NX potentiation of APO stereotypy. On the other hand, pretreatment with reserpine [1.0 mg/kg, ip, 24 hr] reduced brain DA by ∿45% and brain NE by ∿50%, yet there was neither behavioral suppression of APO climbing nor reversal of NX potentiation of APO stereotypy. Such findings may still be consistent with the original hypothesis if reserpine spares functional transmitter pools that are involved in the drug interaction. We are continuing to investigate the underlying mechanism of narcotic antagonist/dopaminergic drug interaction. (Supported in part by research grants from Merck & Company and the Marquette University Committee on Research.)

131.7 INTRACELLULAR STUDIES OF NIGRAL DOPAMINE NEURONS: MORPHOLOGY, ACTION POTENTIAL GENERATION, AND EFFECTS OF APOMORPHINE. A. A. Grace and B. S. Bunney. Depts. Pharmacology and Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06510

Lucifer yellow-injected rat nigral dopamine (DA) neurons demonstrate the following morphology: (1) pyramidal or polygonal shaped somas, 12-30 um in diameter; (2) 3-6 thick (2-6 um dia.) major dendrites extending 10-50 um before dividing; (3) a putative axon arising from a major dendrite 15-30 um from the soma.

DA cell activity consists of four components: (1) a slow, pacemaker-like depolarization (13 ± 3 mV, 60 ± 25 mSec, mean ± S.D.) preceding the spike. This potential is voltage-dependent and consists of a net inward current located at the soma. (2) An initial segment (IS) spike (13-18 mV, 0.5-1 mSec) originating distally from the soma, which can be activated independently of the somatodendritic (SD) spike in the absence of a concurrent slow depolarization (e.g., with antidromic activation). (3) SD spikes (55-75 mV, 1-4 mSec) which originate distally from the soma--most likely in the dendrites. This spike is at least partially calcium mediated, since it is associated with an afterhyperpolarization (AHP) and is sensitive to iontophoretically-applied cobalt. (4) AHP (2.3 ± 0.8 mV) associated only with the SD spike. The amplitude of the AHP is proportional to the number of SD spikes elicited by depolarization. The sequence of these four events would appear to be as follows: A slow inward current at the soma depolarizes the IS to threshold, which then fires a spike. This spike spreads across the depolarized soma to trigger a dendritic calcium spike. A calcium-activated AHP then occurs, which decays to trigger the next slow depolarization.

Intravenous administration of the dopamine agonist apomorphine is known to inhibit firing of DA neurons recorded extracellularly (Bunney, et al., JPET 185:560, 1973). During intracellular recording apomorphine (30-50 ug/kg, i.v.) inhibited all spontaneously firing cells tested (N=10), as well as inactivating both the spontaneously occurring and the depolarization elicited slow depolarizations. An increase in membrane resistance (44 ± 24% increase) concomitant with a hyperpolarization (3.4 ± 1.5 mV) was also observed (N=12). The calculated reversal potential of the apomorphine effect was significantly higher ($p < .001$) for spontaneously firing cells (-41.4 ± 7.0 mV; N=8) than for quiescent cells (-66.3 ± 2.9 mV, N=4). Haloperidol reversed apomorphine's effects in all cells tested (0.1 mg/kg i.v., N=4). Thus apomorphine probably has two effects on DA cells: (1) elimination of the pacemaker current at the soma in spontaneously firing cells, and (2) a change in the conductance of a set of ion channels with a more negative reversal potential. (This work was supported by USPHS grants MH-25642, MH-14276, GM-07527 and the State of Connecticut.)

131.8 BEHAVIORAL AND ELECTROPHYSIOLOGICAL EFFECTS OF INTRANIGRAL APOMORPHINE IN THE MOUSE, F. N. Ross-Cisneros* and P. K. Randall*, (SPON: J. K. Engelhardt) Dept. of Physiology & Biophysics, U. S. C. Sch. of Med., Andrus Gerontology Ctr., Los Angeles, CA 90007

Previous studies have suggested a regulatory role for dopamine in pars compacta of the substantia nigra in controlling nigrostriatal impulse flow through inhibitory "autoreceptors" on nigral cell bodies and dendrites. Though Groves, et al. (Science 190:522-529, 1975) and Aghajanian and Bunney (Advan. Biochem. Psychopharmacol. 16:433-438, 1977) have observed electrophysiological evidence for this hypothesis by demonstrating a decrease in nigral cell activity in pars compacta with local application of dopamine and dopamine agonists in rats, several other investigators have observed neither behavioral nor biochemical evidence for this hypothesis. We have observed both behavioral and electrophysiological evidence that nigrostriatal impulse flow can be inhibited by low doses of intranigral apomorphine.

On-going electrical activity was recorded from bipolar gross electrodes (1/2mm tip separation) from rostral striatum of freely moving C57BL6/J mice prior to and following intranigral cannulation of apomorphine into the ipsilateral substantia nigra. Injections were made through a 30 g injection cannula which was flush with the ventral tip of the 26 g guide cannula previously implanted just dorsal to the body of the pars compacta. Injection was made at the rate of 0.25 µl/30 sec resulting in a total volume of 1 µl at 2 min.

Five, 10 and 50 ng injections resulted in immediate synchronization (increase in amplitude, decrease in frequency) of the striatal potential, accompanied by a severe ipsilateral postural torsion. Both the electrophysiological and behavioral effects were of short duration, usually lasting approximately 30 min post-injection. Higher doses (e.g. 5 µg) resulted in a slight desynchronization of the striatal signal accompanied by ipsilateral postural asymmetry. Behavioral and electrophysiological parameters were unaffected by equivolume injection of vehicle.

Since at the lower doses the behavioral and electrophysiological response was similar to that produced by dopaminergic blockade, these results are consistent with autoregulatory inhibition of impulse flow in DA neurons. The inconsistent response to higher doses suggests either action at a separate lower affinity receptor or a non-specific pharmacological effect of the drug.

131.9 EFFECTS OF CHRONIC NEUROLEPTIC TREATMENT ON NIGRAL DOPAMINE CELL ACTIVITY. L. A. Chiodo and B. S. Bunney. Departments of Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510

We have previously reported that, unlike acute administration, chronic (21 days) haloperidol pretreatment reduces the number of spontaneously active dopamine (DA) neurons present within the rat substantia nigra zona compacta (A9) (Bunney and Grace, Life Sciences 23: 1715, 1978). Moreover, it was inferred that the "silent" DA cells were in a state of tonic depolarization block since they could be induced to discharge by local application of agents which normally hyperpolarize DA cells (e.g. GABA) and not by those which usually depolarize them (i.e., glutamate). In the present study we used extracellular single-unit recording techniques to determine the effects of chronic treatment (21 days, drugs administered orally via drinking water) with a variety of neuroleptics (haloperidol, clozapine, l-sulpiride) and control compounds (promethazine, d-sulpiride, desimipramine) on the spontaneous activity of DA neurons in both A9 and the ventral tegmental area (A10) of anesthetized male albino rats.

Both haloperidol and l-sulpiride significantly decreased the number of spontaneously active cells encountered in A9 and A10 relative to saline controls. However, d-sulpiride, promethazine and desimipramine did not produce this effect. In contrast, clozapine, while producing a similar decrease in the number of active DA cells in A10, caused a significant increase in the number of cells encountered in A9.

These results suggest that chronic oral intake of neuroleptics may increase the activity of both A9 and A10 DA cells to the point that a great majority go into tonic depolarization block. The suggestion that A10 neurons in addition to A9 cells go into depolarization block is supported by the fact that silent A10 cells in haloperidol treated animals could be "activated" by the iontophoretic application of GABA. However, in all of our chronic experiments a small number of DA cells were found to be still active. Studies using antidromic activation to identify the projection area of the cells studied revealed that the great majority of the active neurons project to the prefrontal or cingulate cortex--DA systems known to be devoid of autoreceptors (Bannon et al., this volume).

The apparent neuroleptic induced depolarization block in midbrain DA neurons appears specific for neuroleptics since neither promethazine, d-sulpiride nor desimipramine produced the same effect. The paradoxical effects of clozapine on the activity of A9 DA cells may be related to its lack of neurological side effects. (This work was supported by USPHS grants MH-25642 and MH-14276, NIH Fellowship NS-07136 and the State of Connecticut.)

131.10 MODULATION OF SYNAPTIC TRANSMISSION AND LTP IN RAT DENTATE GYRUS BY STIMULATION IN AND NEAR THE LOCUS COERULEUS. W. C. Abraham and G. V. Goddard. Dept. Psychology, Univ. Otago, Dunedin, New Zealand.

Brainstem nuclei, particularly monoaminergic structures, can have potent influences on synaptic transmission of excitatory pathways in the hippocampus. Stimulation of the locus coeruleus (LC), for example, may increase the population spike (PS) without concomitant changes in the field EPSP of the perforant path (PP) evoked field potentials recorded in the dentate gyrus (DG) (Bliss and Wendlandt, Proc. Int. Union Physiol. Sci. 1977; Assaf et al., J. Physiol. 1979 P; but see Winson, J. Neurophysiol. 1980). In addition, lesions of the ascending noradrenergic pathway from LC impair long-term potentiation (LTP) of the EPSP (Bliss et al., Adv. Physiol. Sci. 1981). We have confirmed that brainstem stimulation in or close to the LC modulates perforant path single evoked potentials . However we have been unable to detect any effects on LTP of these potentials using equivalent brainstem stimulation.

Acute experiments were performed on rats anesthetized with sodium pentobarbital. Field potentials in the DG hilus of each hemisphere were evoked by bilateral diphasic electrical stimulation of the PP. Unilateral brainstem stimulation trains (50 Hz, 10 pulses, 200-300 uA ending 40 msec prior to the PP pulse) produced a parallel shift to the left of the input/output curve relating PS amplitude to EPSP amplitude (i.e. an apparent increased neuronal excitability). Closer analysis revealed not only an enhanced PS (largest voltage change about 45% of control response) but also a diminished EPSP (-15%) for a given stimulus intensity. The shift in excitability occurred bilaterally, although the effect was somewhat greater ipsilateral to the brainstem electrode. Single brainstem pulses had no effect on hilar potentials.

Brainstem stimulation identical to that used to modify single PP evoked potentials and temporally contiguous with high frequency PP trains, (400 Hz, 25 msec), failed to alter the threshold development, asymptotic amplitude or subsequent decay (80 min) of ipsilateral LTP of either the EPSP or the PS. To date we have only employed PP trains embedded within the brainstem trains. We will also report findings from a study in which the brainstem trains trail the PP trains by 200 msec.

Most of the effective brainstem electrode sites (8/11) were histologically verified to be in or near the LC. Further studies are required to determine if the modulation of PP evoked potentials is noradrenergic in nature.

131.11 CONDUCTION PROPERTIES OF LOCUS COERULEUS AXONS IN MONKEY. Gary Aston-Jones†, Stephen L. Foote and Menachem Segal*‡ Center for Behavioral Neurobiology, Salk Institute, San Diego, CA; †Department of Psychology, SUNY at Binghamton, New York; ‡Isotope Department, Weizmann Institute, Rehovot, Israel.

Previous studies have revealed that norepinephrine-containing locus coeruleus (NE-LC) axons in rat conduct impulses slowly (mean velocity about 0.5 M/sec). Such slow impulse conduction in larger brains (as in primates) would result in very long conduction latencies for activity to reach distant target areas (e.g., cerebral cortex), with implications for possible functions of this system. Therefore, using antidromic stimulation techniques, we investigated impulse conduction characteristics of NE-LC axons in a primate brain.

NE-LC neurons were recorded in halothane-anesthetized, adult squirrel monkeys. Spontaneous discharge: These cells discharged spontaneously at about 0.5-1.0 Hz, somewhat slower than in rat (2.4 Hz). Conduction velocity: Monkey NE-LC neurons were often driven antidromically (as verified by collision testing) from the hypothalamus with antidromic latencies most often between 10 and 20 msec, translating to conduction velocities of 0.5-1.0 M/sec. This conduction speed is somewhat faster than that reported for rat NE-LC neurons driven antidromically from the dorsal midbrain noradrenergic bundle (mean values reported = 0.4-0.6 M/sec). Fluctuations in conduction velocity: Antidromic impulses were found to vary in latency as a function of the number and frequency of stimuli presented, such that conduction velocities transiently increased and subsequently exhibited a more pronounced decrease during 10 Hz activation, as previously described for rat NE-LC axons (Aston-Jones et al., Brain Res. 195: 215, 1980). Refractory period: Using double pulse antidromic stimulation, absolute refractoriness occurred for some 1-3 msec following stimulation of an impulse, slightly shorter than in rat. Orthodromic responses: NE-LC neurons were often driven orthodromically from subcutaneous electrical stimulation of the contralateral rear foot. Orthodromic responses to such footshock occurred at latencies of 50-100 msec, somewhat longer than similar responses in rat which occur at 20-40 msec. Post-activation decrease in impulse activity: Both antidromic and orthodromic activation of monkey NE-LC neurons were followed by a prolonged period of decreased activity, as previously reported for rat NE-LC neurons.

Our results indicate that many properties of primate NE-LC neurons are similar to those in rodent, although conduction velocity and recovery from refractoriness appear to be faster. This work was supported by USPHS grants NS 18023 and AA 07273.

131.12 NORADRENERGIC ACTIVATION OF SEROTONERGIC DORSAL RAPHE NEURONS RECORDED IN VITRO. C.P. VanderMaelen and G.K. Aghajanian. Depts. Psychiat. & Pharmacol., Yale Univ. Sch. of Med., New Haven, CT 06508.

Previous studies have shown that 1) spontaneous activity of serotonergic dorsal raphe (DR) neurons in the anesthetized rat is largely dependent upon a facilitatory noradrenergic input, and 2) spontaneous activity is present in the DR nucleus in the brain slice preparation. The present study investigated noradrenergic influences on serotonergic DR neurons in the brain slice. 400 μm-thick slices were cut through the DR nucleus of halothane-anesthetized rats, and placed in a chamber with flowing artificial CSF of the following composition in mM: NaCl, 130; KCl, 6.25; NaH_2PO_4, 1.25; $NaHCO_3$, 24; $CaCl_2$, 2.0; $MgSO_4$, 3.0; D-glucose, 10. Humidified 95% O_2, 5% CO_2 flowed across the slices, kept at 36.5^0 C $\pm$ 0.5^0. Extracellular single unit recordings with single barrel or 5-barrel (iontophoretic) electrodes were made from DR neurons up to 11 h after decapitation.

Many spontaneously firing (i.e. > 0.1 Hz) serotonergic DR neurons with slow, rhythmic firing patterns were encountered in this preparation. However, about half of the viable serotonergic neurons in the slice were not spontaneously active, as seen when the excitatory amino acid glutamate was applied while searching for cells. Virtually all identified serotonergic neurons (both spontaneously active and quiescent) could be excited by small amounts of iontohoretically applied norepinephrine (NE), and by bath application of the alpha adrenoceptor agonist phenylephrine (PE). Neurons were identified as serotonergic if they were inhibited by low currents of iontophoretically applied LSD, as occurs In vivo. Mean discharge rate for spontaneously active serotonergic neurons was 1.5 Hz (N = 31). Acute reserpine pretreatment of rats to reduce endogenous NE resulted in only a slight decrease in spontaneous activity.

Dose-response curves for PE-induced activations of serotonergic DR neurons yielded ED-50 estimates of 2-6 μM ($\bar{X}$ = 4.3 μM). The facilitatory actions of PE or NE could be reduced or blocked by bath application of the alpha-antagonists phentolamine (50 μM) and thymoxamine (15 μm), and by the selective alpha-1-antagonist prazosin (20 nM).

In summary, in this slice preparation approximately half of the viable serotonergic DR neurons fire spontaneously. However, at least some serotonergic neurons In vitro are dependent upon noradrenergic stimulation via alpha-1 receptors to maintain normal spontaneous activity. Supported by Grants MH-17871, MH14276, and the State of Connecticut.

131.13 POTENTIATION OF THE PERFORANT PATH EVOKED POTENTIAL IN THE DENTATE GYRUS BY LOCUS COERULEUS STIMULATION. C. W. Harley, J-C. Lacaille* and S. Milway*. Department of Psychology, Memorial University of Nfld., St. John's, Nfld. A1B 3X9

Two paradigms were used in assessing modulation of the dentate gyrus by locus coeruleus (LC) stimulation in the urethane anesthetized rat (N=35). In one paradigm a brief, 15 msec, 300 Hz train of 20-25 V. 200 μsec square waves to the LC was paired with a 200 μsec perforant path (PP) stimulus of sufficient voltage to evoke a population spike in the dentate gyrus. PP stimuli occured once every 10 seconds and recordings were made in the dentate cell layer. LC stimulation delivered 40 msec prior to PP activation invariably produced a clear enhancement of the dentate population spike averaging 40%-50% and ranging from 15% to 300%. Smaller population spikes were enhanced relatively more than larger ones at the same site.

The short-term enhancement was not seen if the LC-PP interval exceeded 70-80 msec and enhancement was maximal at 30-40 msec intervals. Short-term enhancement was obtained for PP potentials both ipsilateral and contralateral to the LC stimulation. Knife cuts transecting the dorsal bundle and intrahippocampal 6-OHDA, both, eliminated or markedly attenuated short-term enhancement.

In 17/28 animals tested for long-term potentiation following contingent locus coeruleus stimulation significant evoked potential enhancement was observed for 30 minutes or more after termination of 50 pairings of the LC-PP stimuli. In the other 11 animals no long-term potentiation was observed and the PP evoked potential returned to baseline or was transiently depressed following the LC stimulation period.

In the second paradigm an attempt was made to more closely approximate the firing frequencies of LC neurons. In these experiments the 200 μsec LC stimulus was delivered at a 10 Hz frequency throughout the 10 sec intervals prior to each PP stimulus. All PP potentials observed during this noncontingent stimulation were enhanced and upon termination of noncontingent LC stimulation PP potential enhancement invariably continued at the same level or increased transiently. After a 10-60 sec LC stimulation period the PP potential enhancement continued for 5-10 min and occasionally lasted 20-30 min. PP stimulation during the LC train is not necessary for this longer-lasting enhancement to occur.

Computerized measurement of EPSP and population spike amplitudes in both paradigms indicated that typically only the population spike amplitude was significantly enhanced by LC stimulation.

It appears that LC stimulation increases the excitability of dentate cells and that the excitability changes induced can be relatively long lasting.

131.14 MODULATION OF POPULATION RESPONSES BY DOPAMINE IN RAT HIPPOCAMPUS IN VITRO. Valentin K. Gribkoff* and John H. Ashe (SPON: R. Elul). Department of Psychology, Univ. of California, Riverside, CA 92521.

A specific neurotransmitter role for dopamine (DA) in rat hippocampus has been suggested by recent biochemical studies (Bischoff et al, Brain Res., 165:161, 1979; Ishikawa et al, Brain Res., 232: 222, 1982). However, other than the depression of the firing rate of pyramidal neurons demonstrated in early iontophoretic studies, very little is known about the physiological function of DA in the hippocampus (Stefanis, Pharmacologist, 6:171, 1964).

The actions of DA on the population responses of CA1 pyramidal neurons to stimulation of Schaffer collateral-commissural fibers was examined in the rat hippocampus in vitro; drug solutions were slowly infused into the recording chamber via push-pull cannula. In the presence of DA (0.5-2.0 mM), a dose-dependent depression of the population EPSP and spike was observed. DA (1.0 mM) could suppress the population EPSP by as much as 30%, while the effect on the population spike was less pronounced, amounting to about 15%. The depression of both responses can be blocked by the DA-antagonist spiroperidol (8-12 μM).

Following the removal of DA there is a progressive growth of both the population EPSP and spike to an asymptotic level significantly above pre-DA values; the growth of the population spike was more rapid than the growth of the population EPSP. These enhanced post-DA levels were consistently observed and were maintained for periods that could exceed 120 minutes. In contrast, the enhancement began in the presence of DA when the depressive actions of DA were blocked by spiroperidol (8-12 μM); however, the period of enhancement was significantly reduced.

Long-lasting modification of hippocampal neuronal excitability following repetitive stimulation of afferents has been intensively investigated (for review, see: Tsukahara, Ann. Rev. Neurosci., 4: 351, 1981). The mechanism(s) of these modifications, however, has been elusive. Recent reports of long-term modulation by DA and c-AMP of slow postsynaptic potentials in superior cervical ganglion (Libet, Nature, 258:155, 1975; Ashe & Libet, Brain Res., 217:93, 1981), as well as the report of an increase in phosphorylated proteins following the stimulation of afferents in hippocampus (Browning et al, Science, 203:60, 1979), are supportive of an important role for catecholamines in both of these structures. These previous findings, and the present results, suggest a modulatory role for DA in the rat hippocampus. (Supported by NIH grant BRSG-RR07010-16).

131.15 SPONTANEOUS ACTIVITY OF STRIATAL NEURONS FOLLOWING SUB-TOTAL DOPAMINERGIC DEPLETIONS. W.B. Orr, T.W. Berger, E.M. Stricker, M. J. Zigmond. Psychobiology Program, Departments of Psychology and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

Previous studies have shown that the neurotoxin 6-hydroxydopamine (6-HDA) can be used to selectively deplete brain dopamine (DA) content. When 6-HDA is administered intraventricularly in doses producing DA depletions of 40-80%, a gradient of DA loss is observed across the medial to lateral extent of the rat striatum (Onn et al., Soc. Neurosci. Abstr., 8, 1982). Immunohistofluorescence shows the greatest depletion in dopamine levels in medial parts of striatum adjacent to the ventricles, while laterally, DA levels in the striatum remain nearly 100% intact. In this study we compared the effects of subtotal DA-depleting lesions on the spontaneous activity of neocortically driven cells recorded from the medial versus lateral parts of the rat striatum.

Extracellular single unit striatal activity was recorded from animals (N=7) six to ten days following bilateral intraventricular injections of 6-HDA (200 ug/rat) and from unlesioned animals (N=8). Lesioned animals received pretreatments of the NE-uptake inhibitor desimipramine (25 mg/kg, i.p.) within 40 min of the last 6-HDA injection. Cells within the striatum were located by passing glass micropipettes filled with 2M NaCl into either the medial or lateral portions of the striatum (1.0 mm anterior to bregma) while simultaneously stimulating sensory-motor cortex (2.0 mm anterior to bregma, after Schultz & Ungerstedt, Brain Res. 142:357, 1978). Ten minutes of spontaneous activity was measured only from those cells which were cortically driven both before and after recordings. No difference was observed between the spontaneous activity of cells in the medial versus lateral portions of the striatum in either lesioned or unlesioned animals (mean spontaneous rates of units in lesioned animals: medial, M $\pm$ SD = .023 $\pm$ .068, N=19; lateral, .040 $\pm$ .084, N=17; and controls: medial, .011 $\pm$ .025, N=19; lateral, .040 $\pm$ .118, N=24).

Past studies have shown increases in striatal firing following much larger 6-HDA or electrolytic-induced lesions of the dopaminergic nigrostriatal pathway. Evidence from this laboratory using immunohistofluorescence on subtotal DA-depleted brains has consistently shown intact DA-containing terminals within regions adjacent to the recording sites. Thus, under conditions of subtotal DA depletions lesion-induced changes in both pre- and postsynaptic elements (e.g., increased number of DA receptors or increased DA turnover) may be responsible for maintaining control level spontaneous rates.

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131.16 THE EFFECTS OF EPINEPHRINE AND NOREPINEPHRINE ON FROG PRIMARY AFFERENT FIBERS. J.C. Hackman, C.J. Wohlberg* and R.A. Davidoff. Neurophysiology Laboratory, VA Medical Center and Dept. of Neurology, Univ. of Miami School of Medicine, Miami, FL 33101.

Although epinephrine (E) and norepinephrine (NE) and adrenergic receptors are present in the spinal cord, the spinal function of catecholamines is not known. Our present experiments were designed to determine the effects of E and NE on dorsal root (DR) fibers.

We used the isolated, hemisected frog spinal cord continuously superfused with HCO_3^--buffered Ringers at 15°C. Responses were recorded from the DRs using sucrose gap techniques. E and NE (0.1-10 μM, 10-120 sec applications) hyperpolarized primary afferent terminals (0.3-1.5mv) for long durations (up to 6 min). At concentrations > 10 μM these hyperpolarizations were increased in duration and were often followed by a depolarization.

During the hyperpolarization spontaneous DR potentials were depressed. The hyperpolarization appears caused in part by this reduction in spontaneous background DR activity, the latter producing a tonic depolarization of afferent terminals. Thus, both the hyperpolarization and the spontaneous activity were significantly, but reversibly, reduced by TTX (1.25μM), Mn^{++}(1.5mM), mephenesin (1.0mM) and DL-α-aminoadipate (1.0mM).

An increased K^+ conductance also appears responsible for the hyperpolarization since we found the hyperpolarization increased when K^+ was eliminated from the superfusate and reduced when the $[K^+]_o$ was elevated (5mM). In addition, several agents known to affect K^+ conductances--tetraethylammonium (TEA, 0.1mM), 4-aminopyridine (4-AP, 0.1mM) and guanidine (0.1mM)--blocked the hyperpolarization.

The hyperpolarization was not caused by activation of an electrogenic Na^+ pump since ouabain (10 μM) was without effect.

The NE, but not the E, hyperpolarization was blocked by phentolamine (10 μM) indicating that the NE response is mediated by an α receptor. Propranolol (0.1mM) did not block the effects of either NE or E.

The amplitude of dorsal root potentials (DRPs) was augmented during E- and NE-induced hyperpolarizations, but the effect was small (∿10%). Similarly, responses of DRs to GABA were increased only slightly (∿10%).

The depolarization evoked by high concentrations of NE and E was reduced in low Na^+-containing Ringers, suggesting that Na^+ may be responsible for this portion of the potential.

These studies provide further evidence for a transmitter function of catecholamines in the spinal cord. E and NE may have a significant role in modulating sensory input to the spinal cord.

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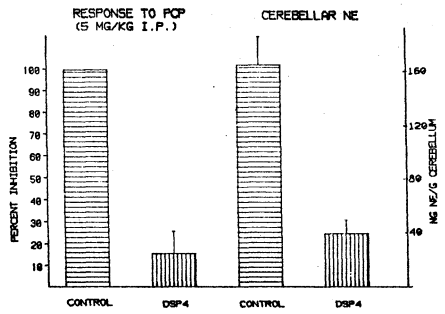
131.17 EFFECTS OF THE NEUROTOXIN DSP4 ON CEREBELLAR PURKINJE NEURON ELECTROPHYSIOLOGY. P.C. Bickford, B.J. Hoffer*, G. Jonsson*†, and R. Freedman*. Dept. of Pharmacology, Univ. Colorado Health Sciences Center, Denver, CO 80262, and †Dept. of Histology, Karolinska Institute, Stockholm, Sweden.

We are currently evaluating DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) as a pharmacological agent to deplete norepinephrine (NE) stores in the CNS. Two to six weeks after an intraperitoneal (i.p.) injection of 50 mg/kg DSP4 or vehicle control, we studied noradrenergic innervation of the rat cerebellar Purkinje (P) neuron using electrophysiological, pharmacological, and biochemical techniques. Phencyclidine (PCP) which acts in the cerebellum primarily via presynaptic NE mechanisms, was used to test the function of the noradrenergic pathway. PCP injected parenterally (5 mg/kg i.p.) decreased P cell firing rates 99.62 ± 0.01% in control rats (N = 4), while depressing discharge only 15.60 ± 10.10% in DSP4 rats (N = 4).



PCP applied locally by micropressure ejection from multibarreled micropipettes produced approximately a 50% decrease in P cell firing rates in all (N = 16) cells recorded from control rats with a mean dose of 24.4 ± 8.86 psi-seconds. Local ejection of PCP was effective in only 25% of P cells (N = 24) recorded from DSP4 rats. The mean dose for responsive cells (N = 6) was 17.0 ± 11.72 psi-seconds, not significantly different from control, which suggested that some NE fibers were still intact. Levels of NE determined by high pressure liquid chromatography were 165.02 ± 19.90 ng/g cerebellum for control and 39.82 ± 8.15 ng/g cerebellum for DSP4 rats, a 75.9% depletion of NE. The data presented here support a physiologically significant destruction of cerebellar NE fibers by DSP4.

(Supported by NS-09199 and DA-02429).

131.18 ROLE OF α_1- AND α_2- ADRENERGIC RECEPTORS IN REGULATION OF EPISODIC GROWTH HORMONE SECRETION. C. Lynch,* R. Free,* C. Longserre,* N. Petersen* and L.C. Terry (SPON: K.L. Casey). Depts. of Neurology and Physiology, Univ. Michigan and VA Hosp., Ann Arbor, MI 48105.

Considerable evidence exists to show that noradrenergic neurons cause release of growth hormone (GH). Recent studies indicate that adrenergic neurons also have a stimulatory influence on GH secretion (L.C. Terry et al., J. Clin. Invest. 69:104, 1982). The effects of these catecholamines on GH release are thought to be mediated by activation of central α_1 and/or α_2-adrenergic receptors (the designation α_1 and α_2 is used here with respect to binding properties rather than anatomical location). The objective of the present studies was to determine the receptor subtype(s) involved in GH regulation by assessing the effects of preferential α_1 and α_2 agents on episodic growth hormone secretion in freely-behaving, chronically cannulated male rats.

Male albino rats bearing chronic indwelling intra-atrial cannulae were kept on a constant light-dark cycle (12/12h with lights on @ 600h) and allowed free access to food and water. Serial blood samples were removed every 15 min. for 3h beginning at 1000h. Drugs or vehicle were administered i.v. between 1005-1010h. The following drugs were tested: yohimbine 0.1, 1.0, 2.5 and 10 mg/kg; rauwolscine 1 and 5 mg/kg; prazosin 0.1 and 1 mg/kg; corynanthine 1, 2 and 5 mg/kg; phenoxybenzamine (an α_1 and α_2 antagonist) 5 mg/kg; and BHT-933 (2-amino-6-ethyl-4.5.7.8-tetrahydro-6H-oxazolo-[4,5-d]-azepin) 0.15, 0.3, .5, 2.5 and 5.0 mg/kg. Yohimbine and rauwolscine are the most selective antagonists of the α_2-adrenoreceptor subtype, whereas, corynanthine and prazosin preferentially block α_1-additional receptors. BHT-933 is a selective agonist of postsynaptic α_2-adrenoreceptors. Yohimbine (1.0-10 mg/kg) and rauwolscine (5 mg/kg) caused a significant reduction in the total amount of GH secreted. (27,219 ± 5660 and 14,905 ± 2394, respectively vs. vehicle control, 48,652 ± 3400 ng/ml x min). BHT-933 caused a significant elevation in mean plasma GH (366 ± 112 vs control, 53.0 ± 20.1 ng/ml). Corynanthine (5 mg/kg) caused a smaller, but significant, reduction in GH (28,754 ± 2673 ng/ml x min). Prazosin had no effect on plasma GH. Phenoxybenzamine also lowered plasma GH (12,656 ± 2896 ng/ml x min).

These results indicate that the stimulatory influence of the central noradrenergic and adrenergic systems on GH secretion is mediated predominantly by activation of postsynaptic α_2-adrenergic receptors. The role of the α_1-adrenoreceptor in control of GH secretion is not clear.

Supported by grants from the VA and NIH.

131.19 CATECHOLAMINE LEVELS IN RATS FOLLOWING MOTOR CORTEX INJURY AND AMPHETAMINE ADMINISTRATION. M.G. Boyeson and D.M. Feeney. Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM 87131.

We have previously found that amphetamine (D-AMP) administered 24 hours after unilateral motor cortex injury in rats and cats accelerates the rate of recovery of motor function (Feeney, D.M. et al., Neurosci. Abst., 6: 1802, 1980: Neurosci. Abst., 7: 930, 1981). To elucidate the mechanisms of D-AMP treatment after injury, levels of catecholamines (CA) were assayed using high pressure liquid chromatography with electrochemical detection. Rats received unilateral motor cortex ablations and at 24 h postinjury received either saline or D-AMP (2 mg/kg, i.p.). Forty-eight h following injury, animals were sacrificed and the levels of CA were assayed in striatum, midbrain, and brainstem. Peliminary results indicate that D-AMP given to uninjured animals significantly reduces CA in all areas compared to saline controls. Interestingly, when uninjured saline controls were compared to injured saline animals, injured saline animals showed a significant increase in norepinephrine (NE) in all brain areas, but no significant change in dopamine. When uninjured animals given D-AMP were compared with injured animals given D-AMP, a significant reduction in CA occurred in the striata of injured animals with no statistically significant changes in CA in other brain areas. Since NE is elevated in all brain areas of injured animals given saline, and since the blood-brain barrier is sealed at 24 h after injury in the rat, the results suggest that the elevated NE levels are endogenous rather than blood-born. Currently, we are investigating hemispheric differences in CA levels in response to motor cortex injury.

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131.20 URINARY CATECHOLAMINE LEVELS IN RATS EXPOSED TO 60-Hz ELECTRIC FIELDS. D. I. Hilton* and L. E. Anderson. Biology Department, Battelle, Pacific Northwest Laboratories, Richland, WA 99352

Recent studies in several laboratories suggest that the nervous system may be influenced by high-strength 60-Hz electric fields. Observed changes in various endocrine and neuroendocrine parameters, as well as alterations in the excitability of synapses in exposed animals, indicate that electric fields may supply sensory input to the peripheral and central nervous systems. To facilitate the investigation of these effects, possible changes in adrenergic sympathetic nerve and adrenal medullary hormone activity were examined by measuring free, unconjugated urinary catecholamine levels in male Sprague-Dawley rats exposed to 60-Hz electric fields.

Ten rats were exposed for 20 hr/day to an unperturbed electric field of 65 kV/m for 30 days. Ten additional animals served as sham-exposed controls. Body weights, 24-hr urine volumes, urinary epinephrine, and norepinephrine levels were measured before exposure and at weekly intervals during exposure. After 30 days exposure, the mean urine volume of the exposed group was significantly lower compared with that of the sham-exposed controls. In addition, the mean values for urinary norepinephrine in the exposed group (0.94 µg/24 hr) were slightly lower than those of controls (1.2 µg/24 hr), although the difference was not statistically significant. There were no differences in urinary epinephrine levels between exposed (0.32 µg/24 hr) and sham-exposed controls (0.28 µg/24 hr). The decreased urinary output observed in the exposed animals may be the result of neuronal and/or endocrine changes due to electric field exposure. Experiments are in progress to resolve this question.

131.21 ^{3}H-SPIPERONE BINDING IS INCREASED IN STRIATAL MEMBRANES OF ADULT MALE RATS. H.R. Wagner, E. Yablonskaya-Alter*, A. Reches, S. Fahn, Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York

Alterations in the density of ^{3}H-spiperone (SPIP) membrane binding sites in the rat striatum have been reported following injection of rats with high doses of estrogen. To establish if such effects occur under more physiological conditions, we have compared ^{3}H-SPIP membrane binding in striata from mature (45-50 day) male (M) and female (F) rats. We have also measured binding in age-matched females ovariectomized (OVX) four weeks earlier.

Striatal binding, assessed at a single concentration of ^{3}H-SPIP (0.2 nM), was significantly lower in membranes from intact female rats relative to male rats ($p \leq 0.05$). Binding compared between male and OVX female rats did not differ significantly (M=212±7; F=186±9; OVX=206±10 fmols ^{3}H-SPIP/mg protein). As determined by Scatchard analysis, the M vs. F difference reflected a lower maximum density (Bmax) of striatal binding sites in intact female rats. Kds were similar (0.1 nM) between the three groups.

Attempts to alter ^{3}H-SPIP striatal membrane binding by administering estrogen to male or OVX female rats were unsuccessful. No changes in the density of ^{3}H-SPIP striatal binding sites were seen in OVX rats injected one or four days earlier with 17-β estradiol benzoate (EB) (125 ug/rat, s.c.) in sesame oil. Binding was also unaffected in male rats injected four days earlier and in OVX females chronically exposed to EB for two weeks through implants of EB-containing silastic capsules (5 mm).

Our failure to alter the density of ^{3}H-SPIP membrane binding with estrogen suggests that the decreased density of ^{3}H-SPIP membrane binding sites in the female rat brain may not be estrogen dependent.

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131.22 ANTI-WITHDRAWAL EFFECTS OF ALPHA METHYL DOPA AND CRANIAL ELECTROTHERAPY. M.S. Gold[1], A.L.C. Pottash[1], H. Sternbach*[1], J. Baraban*[2], W. Annitto*[1]. [1]Fair Oaks Hospital, Summit NJ, [2]Columbia University School of Medicine, NY NY. We have investigated the neural events which follow the discontinuation of chronic opiate administration and result in clinical signs and symptoms in man. We have reviewed the rodent, primate and human data which have supported an endorphin-locus coeruleus (LC) disinhibiton hypothesis and a noradrenergic (NE) neuroanatomy for opiate withdrawal (1). Using this (NE) hypothesis to test new compounds has lead to the discovery of two nonopiate treatments for drug withdrawal and naturally occurring panic and anxiety states-clonidine and lofexidine (2,3). Since clonidine, by stimulating brain alpha-2 adrenoceptors, decreases central noradrenergic neuronal activity we have postulated that the inhibitory effect of clonidine on noradrenergic transmission underlies its efficacy in the treatment of opiate withdrawal. We now have data from humans in acute and protracted opiate withdrawal which demonstrates significant antiwithdrawal efficacy for transcutaneous cranial electrical nerve stimulation (T.E.N.S.;C.E.S.) and the antihypertensive agent alpha methyl dopa which support this NE theory on the basis of endorphin and alpha adrenergic inhibition of the LC.

Subjects who have been chronic opiate users, who were otherwise in good health as demonstrated by a comprehensive medical and psychiatric evaluation in the hospital, were maintained and studied in Fair Oaks Hospital as reported previously (1-3). 48 hours after abrupt methadone discontinuation they were given a 500 mg p.o. test dose of alpha methyl dopa (Aldomet) and placebo in a randomized order, double blind. Aldomet but not placebo had significant acute antiwithdrawal activity. A second group of similar subjects were treated with placebo electrotherapy and/or electrotherapy. Cranial stimulation, but not placebo electrotherapy, had significant acute antiwithdrawal efficacy and efficacy in protracted withdrawal.

The functonal integrity of the B-endorphin system which sends long axons from a cell group near the arcuate nucleus to the LC may be compromised by chronic self-administration of potent exogenous opiates. Acute and protracted opiate withdrawal may result from a sudden absence of exogenous opiates and an inadequate functional endorphin reserve allowing the release of previously inhibited NE systems and clinical signs and symptoms. The efficacy of alpha methyl dopa and electrotherapy in acute withdrawal and electrotherapy in protracted withdrawal in man may be due to anti-NE effects of alpha methyl dopa and endorphin mediated anti-LC effects of electrotherapy.

1. Gold, M.S., et al Biomedicine, 3:1-4,1979.
2. Gold, M.S., et al N Eng J Med, 302:1421-1422, 1980.
3. Gold, M.S., et al Adv in Alco and Sub Abuse 1:33-51, 1981.

131.23 ROLE OF ALPHA-2 ADRENERGIC MECHANISMS IN NOCICEPTION. D. R. Haubrich, D. Mastriani*, R. Ferraino*, R. Ferrari*, M. Perrone*, L. Trethaway*, T. Knapp* and A. Burns*. Neuropsychopharmacology Section, Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, NY 12144.

Clonidine (Cl), guanabenz (GB), guanfacine (GF), BHT-920 and xylazine (X) were used to assess the role of alpha-2 adrenergic mechanisms in nociception. The alpha-2 adrenergic agonist activity of these compounds was demonstrated in vitro by their ability to inhibit the twitch height of the field-stimulated rat vas deferens (IC_{50}'s from 0.6 (GF) - 14 (X) nM) and to prevent the binding of ^{3}H-clonidine to membranes isolated from rat brain (K_i's from 1 (GB) to 70 (X) nM).

When administered to mice, all five compounds displayed antinociceptive activity (phenyl-p-quinone writhing test), with ED_{50}'s that ranged from 20 (C) to 900 (X) µg/kg (s.c.). In addition, treatment of mice with these alpha-2 agonists caused them to fall off a rotorod, but the doses required were 2-15 times greater than the antinociceptive doses, indicating that antinociception was not the result of generalized CNS depression.

Pretreatment of mice with yohimbine (1 mg/kg, p.o.) to selectively block alpha-2 receptors antagonized the antinociceptive effect of all five compounds, and shifted the dose-response curves by a factor of 4-6. Clonidine-induced antinociception was also antagonized by pretreatment with the selective alpha-2 adrenergic antagonists piperoxan, tolazoline or rauwolscine, but not by the alpha-1 adrenergic antagonists phentolamine, prazosin, WB4101, mianserin, trazodone or corynanthine. Pretreatment of mice with antagonists of opiate, dopamine, serotonin, histamine, β-adrenergic or cholinergic receptors had no effect on clonidine-induced antinociception. These results support the hypothesis that alpha-2 adrenergic mechanisms are involved in regulation of the nociceptive threshold, and suggest that selective alpha-2 adrenergic agonists may be analgesics.

131.24 PROLACTIN STIMULATES DOPAMINE RELEASE FROM MALE, BUT NOT FROM FEMALE RAT STRIATAL TISSUES SUPERFUSED IN VITRO: Yiu-Fai Chen and Victor D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801

Direct prolactin effects on dopamine (DA) release from fragments of rat striatal tissue were studied by the in vitro superfusion technique. Intact and gonadectomized (1 week prior the experiments) adult male and female Holtzman rats were killed by decapitation at 1000-1100 h (under a 0500-1900 h light schedule). Proestrous females were killed at 1500. Striatal tissues were quickly dissected out and placed in cold Krebs-Ringer-phosphate buffer (pH 7.4) containing glucose(10 mM), bovine serum albumin (0.1%), ascorbic acid(0.1%) and pargyline(3.5×10^{-4} M). This same buffer was also used for superfusion of the tissue. The equivalent of 2 rat striatal fragments were placed in each experimental chamber and allowed to stabilized for 60 min. The chamber was positioned in a constant 37° C water bath. The medium volume in the chamber (500 ul) was replaced at a constant flow rate of 125 ul/min. Effluent samples were then collected on ice in 0.1 N $HClO_4$ at 4 min intervals. The DA collected in the superfusates was quantified by a radioenzymatic assay.

The spontaneous release of DA from male and female rat striatal tissues were relatively stable. The basal release rate of DA during the first 16 min of superfusion were (1) intact male, 25.3 ± 1.5 (n=25 experiments); (2) castrated male, 23.3 ± 3.9 (n=8); (3) diestrous I female, 24.4 ± 1.6 (n=12); (4) ovariectomized female, 25.0 ± 3.3 pg/mg/min (n=10). However, the basal DA release rate from striatal tissue from proestrous females was significantly higher ($p < 0.01$) than the other groups (73.8 ± 14.4 pg/mg/min, n=5).

Infusion of prolactin (NIAMDD-rPRL-I-5) in doses of 1 ug/ml or 10 ug/ml for 24 min significantly increased DA release from striatal tissues from intact males (167 ± 15% (6) and 161 ± 34% (7) over pre-infusion levels, respectively). In castrated males the increase was 203 ± 32% (n=4, PRL 1 ug/ml). Lower doses of prolactin (0.01 ug or 0.1 ug/ml) had no effect on DA release from striatal tissues from either intact or castrated males. DA release from striatal tissues from intact males was not altered by heat-denatured prolactin (1 ug/ml) infused for 24 min or other proteins. In parallel experiments, infusion of prolactin at 0.01, 0.1, 1, and 10 ug/ml levels for 24 min had no effects on the basal release of DA from striatal tissues from intact or ovariectomized females.

These results demonstrate that an absolute sexual difference exists in the responsiveness of rat striatal tissues to prolactin infused in vitro. Interestingly, this sexual difference was not modified by short-term castration. (Supported by a NIH grant HD 14625 to VDR.)

131.25 EXCITATORY INPUT FROM FRONTAL CORTEX TO CAUDATE PUTAMEN AND ITS MODIFICATION BY CONDITIONING STIMULATION OF THE SUBSTANTIA NIGRA. K. Hirata*, C. Y. Yim* and G. J. Mogenson. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

The caudate putamen (CP) is known to receive an excitatory input from the cerebral cortex as well as a dopaminergic (DA) input from the substantia nigra pars compacta (SNc). The present study investigated possible interaction of these two inputs.

Extracellular single unit recordings were obtained from 215 neurons in the CP of urethane-anesthetized rats using glass micropipettes filled with 0.5 M sodium acetate. Most CP neurons had slow discharge rates of 4-8 spikes/sec. Stimulation of the FC resulted in excitation of 61% of CP neurons with a mean onset latency of 8.3 ms. Conditioning stimulation of the SNc with a train of 10 pulses (400 uA, 0.15 ms delivered at 10 Hz) 1100 ms before single pulse stimulation of FC modified response of CP neurons to FC stimulation. In 12 cases, the response was attenuated and in 5 cases, the response was enhanced. Intraperitoneal injection of haloperidol, a DA antagonist, reduced both attenuating and enhancing effects of SNc conditioning stimulation.

These observations confirm results from previous reports that the cerebral cortex has a strong excitatory input to the CP. In addition, conditioning stimulation of the SNc modulated these responses. The modulating effect of SNc stimulation was likely due to release of DA from activation of DA neurons of the SNc since haloperidol reversed the effect. It was previously observed that DA released in the nucleus accumbens (NA) modulated the excitatory response of NA neurons to amygdala stimulation (Yim and Mogenson, Brain Res. 1982, in press). The similarities and differences between the modulatory effects of DA release in NA and CP, as well as the significance of this modulatory role of DA in relation to the function of the basal ganglia are being investigated.

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131.26 NOREPINEPHRINE-INDUCED CYTOCHROME c OXIDASE REDOX RESPONSES AND HEMODYNAMICS IN RAT CEREBRAL CORTEX DURING NORMOXIA AND HYPOXIA. A.L. Sylvia and C.A. Piantadosi*. Departments of Physiology and Medicine, Duke University Medical Center, Durham, N.C. 27710.

Differential reflectance spectrophotometry was used to simultaneously monitor in vivo changes in cerebral cytochrome c oxidase (Cyt. a,a_3) redox states, hemoglobin saturation (Hb/HbO_2), and relative regional blood volume (rBV) in response to systemic norepinephrine (NE) administration. Absorbance changes were measured in parietal cortex through the skull intact rat brain using sample minus reference light (λS-λR) wavelength pairs of 605-590nm (Cyt.a,a_3), 577-586nm (Hb/HbO_2), and at 586nm (rBV). The effects of i.v. norepinephrine (NE) administration (0.125-4.0µg/kg) on the above parameters were measured under normoxic (F_iO_2 0.30; PaO_2 100 mmHg or above) and hypoxic (FiO_2 0.12; PaO_2 30-35 mmHg) conditions. Each animal served as its own control; and while there was considerable inter-individual variability, NE administration during normoxia produced dose-dependent transient increases in the level of oxidized Cyt. a,a_3 accompanied by slight increases in hemoglobin saturation. These responses were followed by disoxygenation concomitant with increases in relative regional blood volume. The above alterations occurred simultaneously with increases in systemic blood pressure (BP). Under hypoxic conditions, low doses of NE promoted moderate increases in BP without changes in the metabolic (Cyt.a,a_3) or hemodynamic (Hb/HbO_2; rBV) signals. At higher doses (1.0-4.0µg/kg), regional blood volume increased but the metabolic signal was reversed (i.e. transient Cyt.a,a_3 reduction) and was accompanied by significant decreases in hemoglobin saturation. The transient oxidative response of Cyt.a,a_3 was restored upon normoxic recovery and significantly enhanced, i.e. greater level of oxidation than normoxic "steady state" conditions, by hyperoxia (FiO_2 1.0). Since it has been shown that the Cyt.a,a_3 redox state in vivo is labile to changes in O_2 supply mediated by cellular metabolic demands, a reductive redox shift indicates insufficient oxygen for cerebral metabolism. In this study, the NE-induced transient oxidative metabolic response is consistent with a passive increase in O_2 delivery to the cerebral tissues due to increased cardiac output, an enhanced cerebral metabolic activity (mitochondrial respiratory state 4-3 transition) or a combination of the above. Results from the hypoxic/hyperoxic experiments appear to support a direct metabolic effect of NE, since at high doses it was possible to reverse the cerebral Cyt.a,a_3 redox transient by limiting oxygen supply.

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132.1 REGULATION OF NEURONAL NOREPINEPHRINE UPTAKE SITE BY IONS AND CHRONIC DRUG TREATMENT. J. A. Javitch*, C.M. Lee and S.H. Snyder. Johns Hopkins University School of Medicine, Depts. of Neuroscience, Pharmacology and Psychiatry, Baltimore, MD 21205.

A variety of evidence indicates that high-affinity [^{3}H]desipramine (DMI) binding in rat brain membranes is associated with norepinephrine (NE) neuronal uptake sites (Lee and Snyder, Proc. Natl. Acad. Sci. USA, 78:5250-5254, 1981; Lee, Javitch and Snyder, J. Neurosci., in press, 1982). Further support for this association is provided by manipulation of adrenergic function with reserpine (which depletes NE from storage granules) and iproniazid (an MAO inhibitor). Chronic reserpine administration (0.5 mg/kg, i.p., 4-18 days) depletes over 90% of the cortical NE content and produces a concomitant decrease in both NE uptake (70%) and high-affinity [^{3}H]DMI binding (20-30%). Also associated with these changes is an increase (20-60%) in β-adrenergic receptor labeled with [^{3}H]dihydroalprenolol (DHA). In contrast, chronic administration of iproniazid (20 mg/kg, i.p., 18 days) doubles the control NE level and is accompanied by an increase in high-affinity [^{3}H]DMI binding (30%) and a decrease in [^{3}H] DHA binding (20%). These findings suggest that like postsynaptic adrenergic receptors, the presynaptic NE neuronal uptake mechanism is also regulated by the state of adrenergic function.

Like NE uptake, high-affinity [^{3}H]DMI binding is dependent on both sodium (Na^+) and chloride (Cl^-). The NaCl stimulation of [^{3}H]DMI binding is due to an apparent increase in B_{max} with no affinity change. Because DMI is not transported by the NE uptake site but merely blocks this process, its recognition by the uptake site may be different than NE's. For this reason we have examined the ability of NE to displace high-affinity [^{3}H]DMI binding at varying NaCl concentrations. At 120 mM NaCl, 8 μM NE displaces 50% of [^{3}H]DMI binding (IC_{50}). The IC_{50} for NE is reduced 10-fold at 50 mM but elevated several fold at 300 mM NaCl. This suggests that NE's affinity for the uptake site may be increased at lower salt concentrations. Attempting to reconcile this observation with the fact that NE uptake is reduced at low salt concentrations may provide direction in elucidating the ionic mechanism of the NE neuronal uptake process.

132.2 ^{3}H-XYLAMINE ACCUMULATION BY THE NOREPINEPHRINE UPTAKE SYSTEM. J.B. Fischer, R.W. Ransom*, L.A. Waggaman*, and A.K. Cho*. Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

Xylamine (N-2'-chloroethyl-N-ethyl-2-methylbenzylamine) (XYL) has been shown to be a specific, irreversible inhibitor of neuronal norepinephrine (NE) uptake in several tissues (Cho et al., J. Pharmacol. Exp. Ther. 214:324, 1980; Dudley et al., ibid. 217:834, 1981; Fischer & Cho, ibid. 220:115, 1982). XYL's effects appear to depend upon its interaction with a functional NE uptake carrier, as its irreversible inhibition does not develop under conditions where NE uptake is inhibited, such as in the presence of cocaine, amphetamine, ouabain, or low [Na+] medium. To further characterize XYL's interaction with the NE uptake carrier, we have examined the accumulation of tritium-labeled XYL in several tissues which take up NE.

(Phenyl-^{3}H)XYL was synthesized in this laboratory and recrystallized to a constant specific activity of 3 Ci/mmol. Tissues were incubated in Krebs-Ringer bicarbonate buffer with ^{3}H-XYL which had been allowed to form its aziridinium ion (Ransom et al., Mol. Pharmacol. 21:380, 1982). After exposure to ^{3}H-XYL, tissues were rinsed, then dissolved in tissue solubilizer, and the tritium was determined by scintillation counting.

In organ-cultured rat superior cervical ganglia (SCG) the accumulation of ^{3}H-XYL, at concentrations below the IC_{50} for NE uptake inhibition, was reduced by 77% when Na+ was replaced by Li+ in the incubation medium, similar to the reduction seen in NE uptake. 1 uM desipramine (DMI) and 10 uM d-amphetamine, both inhibitors of neuronal NE uptake, decreased ^{3}H-XYL accumulation by 78%. Cocaine inhibited accumulation with an IC_{50} of about 1 uM, which is similar to that for cocaine's inhibition of NE uptake. In rat vas deferens, ^{3}H-XYL accumulation was reduced by 56% in the absence of Na+, and 45% in the presence of 100 uM NE. ^{3}H-XYL accumulation in rabbit thoracic aorta was also significantly decreased by DMI and by the absence of Na+ in the incubation medium. In all tissues tested, accumulation was decreased by over 90% at 0°C. ^{3}H-XYL accumulation in vas deferens was found to plateau between concentrations of 1 and 5 uM, concentrations which maximally inhibited NE uptake. Similarly, in SCG, accumulation plateaued at concentrations which significantly inhibited NE uptake.

In conclusion, the accumulation of ^{3}H-XYL into tissues showing neuronal NE uptake appears to be dependent upon the NE uptake carrier. Additionally, this carrier-mediated accumulation may be related to XYL's inhibition of NE uptake, since the sensitivities of these two processes to various manipulations are similar. (Supported by USPHS Grant MH23839.)

132.3 THE RATE OF DOPAMINE TURNOVER IS HIGHER IN MESOLIMBIC THAN NIGROSTRIATAL NEURONS. C.H. Cheng and G.F. Wooten, Depts. of Neurol. and Pharmacol., Washington Univ. Sch. of Med., St. Louis, MO 63110

Dopaminergic cell bodies in the substantia nigra (A-8,9) project to the corpus striatum (CS) thereby forming the nigrostriatal pathway; while more medially located dopaminergic neurons in the ventral tegmental area (A-10) project to a variety of limbic structures including nucleus accumbens and olfactory tubercle thereby forming the mesolimbic pathway. Both anatomical and biochemical studies suggest that there are differences in the control of dopamine (DA) metabolism between nigrostriatal and mesolimbic neurons.

To determine if there are differences in DA turnover between nigrostriatal and mesolimbic neurons, we have estimated DA turnover by two independent methods in the CS and in a dissected region of the brain containing the olfactory tubercle and nucleus accumbens (OA). DA and its metabolites were separated and quantified by high performance liquid chromatography with electrochemical detection (LCEC). Rats were treated either with saline or the tyrosine hydroxylase inhibitor, alphamethylparatyrosine (AMPT) 400 mg/kg intraperitoneally. Two hours later the rats were killed and the DA content of the CS and OA was determined. AMPT treatment resulted in a 38% reduction in DA content of the CS and a 53% reduction in the OA ($P < 0.05$).

DA turnover was also estimated by injecting tracer doses of tritium labeled L-DOPA (200 μCi; approximately 7 μg/kg) intravenously in carbidopa (25 mg/kg I.P.) pretreated rats and determining the distribution of tritium among DOPA, DA, DOPAC, and HVA in homogenates of OA and CS at various time points. Biochemical separation and identification of the compounds was accomplished by LCEC. The ratios of tritium-labeled DA to DOPAC plus HVA in CS and OA were as follows: at 5 min, CS 9.9 and OA 6.4; at 25 min, CS 5.5 and OA 2.4; at 45 min, CS 4.5 and OA 1.7; at 2h, CS 3.1 and OA 1.4; and at 4h, CS 2.3 and OA 1.1.

Taken together these results suggest that the turnover rate or rate of synthesis and release of DA is higher in mesolimbic than in nigrostriatal neurons. These regional differences in DA turnover may result in regional differences in the effects of certain drugs that affect DA metabolism.

132.4 IN VIVO ESTIMATION OF SPONTANEOUS AND AMPHETAMINE STIMULATED DOPAMINE RELEASE FROM RAT STRIATAL TISSUE USING PUSH-PULL PERFUSION (PPP) AND HPLC-EC. J.C. Chen, K.K. Rhee, and V.D. Ramirez. Dept. of Physiology and Biophysics, University of Illinois, Urbana, Il. 61801.

Previously we reported sex-differences in the AMPH-evoked DA release from rat striatal tissue superfused in vitro (Becker & Ramirez, Brain Res. 204:361, 1981). We have applied the PPP (Levine & Ramirez, Endocrinology 107:178, 1980) concomitantly with High Performance Liquid Chromatography with Electrochemical detector using a Biophase column (OSD-18C, 5 um spherical particles) as stationary phase and 0.1M citrate/acetate buffer, pH 5.1 as mobile phase to estimate DA output from rat striatal tissue in conscious, freely moving female rats. In the present study, we describe a simple procedure to measure DA and other metabolites in untreated perfusate samples. To validate the method, rostral striatal tissues (CS) from decapitated male rats were quickly dissected out and placed in cold superfusion medium (modified Krebs-Ringer-Phosphate medium, pH 7.4). Within 30 min the tissues were homogenized in cold superfusion medium, centrifuged in a J-21-B centrifuge at 5000 rpm for 25 min using a JA-14 rotor and the clear, untreated supernatants immediately were injected into the HPLC. The DA concentration in these CS was 4.4 ± 0.17 ng/mg wet tissue ($\bar{x}$ ± SE, n=4). After 4h there was little degradation of DA in samples kept at 0-4 C. When a known amount (200 pg) of freshly prepared DA was added to each tissue samples, the % recovery was 99 ± 4.9% (n=4). With this simple procedure dopamine, 5-hydroxy-indole-acetic acid and homovanillic acid were eluted at 5, 13.4 and 17.2 min after the injection. Normetanephrine and dihydroxy-phenylalanine were coeluted 3.6 min post-injection, and 5-hydroxytryptophan and metanephrine co-chromatographed at 7.8 min. A perfect linear dose-response between 30 and 1000 pg of DA dissolved in medium was also demonstrated.

Push-pull cannulae were then implanted into the rostral corpus striatum of mature female rats. Ten-twenty days following implantation, the rats were subjected to in vivo push-pull perfusion at a slow rate of 20-25 ul/min with medium and the samples were collected on ice at 20 min intervals. Following a baseline collection period, d-AMPH sulfate (10^{-3}M in medium) was infused for a 20 min period. Immediately upon collection, 200 ul of each sample was injected into the HPLC. The basal release rate of DA from the CS of diestrous female rats was 5.42 ± 1.29 pg/min; the highest DA value obtained after AMPH stimulation was 30.37 ± 5.17 pg/min (n=3). These data demonstrate that it is feasible to estimate the output of DA and other metabolites in untreated perfusate samples from localized CS tissue of conscious rats using a PPP in line with a HPLC-EC system. (Supported by NIH grant HD-14625 to VDR.)

132.5 THE RELATIONSHIP BETWEEN SAM-DEPENDENT METHYLATIONS, CYCLIC-AMP LEVELS AND DEPOLARIZATION-DEPENDENT NEUROSECRETION FROM PC12 CELLS. R. McGee and C. S. Rabe*. Dept. of Pharmacol. Georgetown Univ., Washington, D.C. 20007

Our previous studies demonstrated that elevation of the cAMP level in PC12 cells by exposure to adenosine or forksolin (a direct activator of adenylate cyclase) caused an enhancement of depolarization-dependent neurosecretion. This enhancement was Ca^{++}-dependent and seen regardless of the nature of the depolarizing stimulus (K^+, carbachol or veratridine) or the neurotransmitter released (norepinephrine or acetylcholine). We also saw that an inhibitor of SAM-dependent methylation, 3-deazaadenosine (3-DA), enhanced depolarization-dependent secretion in a manner analogous to that produced by elevated cAMP. In attempting to determine if the effects of 3-DA were related to inhibition of methylation, the rate of phospholipid N-methylation and protein carboxymethylation in intact cells was determined using intact cells and ^3H-CH_3-methionine. 3-Deazaadenosine inhibited both methylation pathways, but the inhibition was observed at concentrations of 3-DA 10-100 times lower than those which enhanced release. Another potent inhibitor of methylation, periodate-oxidized adenosine, (Arch. Biochem. Biophys. (1980) 205, 132) also inhibited methylation at concentrations 10-100 times lower than those which had any effect on secretion. These data, along with our previous observation that the rate of methylation does not change during secretion, provide reasonably conclusive evidence that methylation and neurosecretion are not directly linked.

Since the enhancement of secretion by 3-DA resembled that seen with elevated cAMP, the effects of depolarization and 3-DA on cellular cAMP were studied. Depolarizing stimuli caused small increases (2-3 fold) in cellular cAMP. These increases were blocked by the absence of extracellular Ca^{++} and antagonists of depolarization. The increases in cAMP, however, were very small compared to the 15-50 fold increases in cAMP produced by concentrations of forskolin or adenosine which enhanced neurosecretion. In the absence of depolarization, 0.3 mM 3-DA, which caused a marked enhancement of secretion, caused only a very small increase in cAMP. However, when cells were exposed simultaneously to 3-DA and depolarization a very large synergistic increase in cAMP was produced. The level of cAMP achieved was similar to that produced by concentrations of forskolin which enhanced release. Thus, as has been shown by Zimmerman et al. (Proc. Nat. Acad. Sci. (1980) 77, 5639), 3-DA not only inhibits methylation but also can act like a phosphodiesterase inhibitior, amplifying the stimulation of cAMP production which occurs during depolarization. It is this latter effect which best accounts for enhancement of depolarization-dependent secretion from PC12 cells. Supported by NIH grant NS 16777 and RCDA NS 00567.

132.6 TEMPERATURE DEPENDENCE OF SECRETION FROM CULTURED CHROMAFFIN CELLS. L.-S. Kao* and E. W. Westhead. Department of Biochemistry, Univ. of Massachusetts, Amherst, MA 01003.

In order to begin to isolate some of the stages in the process of exocytotic secretion, we have examined the rate of secretion of catecholamines by cultured bovine chromaffin cells over a temperature range of 4° to 37°C. Chromaffin cells were isolated and cultured by the methods of Kilpatrick et al. (J. Neurochem. 35, 679, 1980). Cells which were kept in culture for 3 to 4 days were used for this study. Epinephrine (E) and norepinephrine (NE) were determined by HPLC with electrochemical detection. The cells were stimulated with acetylcholine (Ach) or 56 mM K^+ in phosphate buffered Locke's solution and both E and NE secretion were followed as a function of time until the maximum secretion was reached (up to 30 minutes). The percentage of E secreted was always lower than that of NE when the cells were stimulated with either Ach or high K^+ at any temperature. When the cells were stimulated with Ach or carbachol the maximum percentage of total E or NE secreted increased with temperature from 4° to 24°C and then decreased from 24° to 37°C. Potassium-stimulated cells secreted increasing amounts of catecholamine as temperature was increased until a plateau was reached at about 30°C. This corresponds with the data by Knight (J. Physiol. 298, 41P, 1979) for total catecholamine at a single time point. However, when we examined the initial rates of secretion rather than the total secretion, we found that the secretion rates increased continuously as temperature increased throughout the range for both carbachol and K^+ stimulated cells. The Arrhenius plots of initial rates show an inflection point at approximately 17°C for carbachol stimulated cells. The slope above 17°C corresponds to an activation energy (E_a) of 10 kcal/mol while the slope below 17°C corresponds to an E_a of 27 kcal/mol. The plot for K^+ stimulated cells is a straight line over the entire temperature region giving an E_a of 20 kcal/mol. Apparently the transition around 17°C is associated with some steps between the binding of acetylcholine to the receptor and depolarization of the cell. The temperature range over which the transition for Ach stimulation takes place is very imprecisely defined but suggests a ΔS for the transition of not less than 180 E.U., which reflects a substantial cooperative transition. (Supported by USPHS grant #GM24197 and by a Biomedical Research Support Grant.)

132.7 RELEASE OF ENDOGENOUS CATECHOLAMINES FROM RAT BRAIN. Charles W. Bradberry* and Ralph N. Adams (SPON: R. T. Borchardt). Dept. of Chem., Univ. of Kansas, Lawrence, Kansas 66045.

Release of catecholamines from in vitro preparations of CNS tissue is generally followed using radiolabeled compounds newly taken up or synthesized. In addition to the expense involved in these techniques, it has been shown that distinct storage pools exist which are selectively labeled. Since release from some pools appears to occur preferentially over others, that "functional pool" which represents the sum of release from the different pools remains obscured. Following the release of endogenous compounds should overcome this biasing. While endogenous release of catecholamines from incubated tissue can be assayed by chromatography, reuptake can compete for release. To avoid the above problems, we have devised a system for studying the release of endogenous catecholamines from minced tissue in a flowing stream. This is an extremely sensitive technique based on the principles of flow injection analysis and utilizing the standard electrochemical equipment used for detection in liquid chromatography. Amounts released (over a 1-2 minute period) as small as one picomole can be detected. Ca^{2+}-dependent K^+-stimulated release of DA from rat striatum and NE from rat thalamus have been observed.

132.8 ALPHAMETHYLTYROSINE ATTENUATES AND RESERPINE POTENTIATES METHAMPHETAMINE-INDUCED NEURONAL CHANGES G. C. Wagner, J. B. Lucot, C. R. Schuster, and L. S. Seiden. Depts. of Biopsychology and Pharmacological and Physiological Sciences, Univ. of Chicago, Chicago, Il 60637

The repeated administration of methamphetamine to rats has been shown to cause long-lasting depletion of dopamine in various brain regions. The interactions of alphamethyltyrosine (AMT) and reserpine with the methamphetamine-induced dopamine depletion were examined in one study. In a second study, the effects of AMT and reserpine on central dopamine levels were measured in rats previously treated with methamphetamine. It was observed that pretreatment with AMT attenuated the long-lasting (1 month) dopamine depletion induced by methamphetamine, whereas, pretreatment with reserpine potentiated the depletion. However, the effects of AMT and reserpine on brain dopamine were not altered when these agents were administered 1 month after the last methamphetamine injection. These results are discussed with reference to the effects of methamphetamine, AMT and reserpine on the vesicular versus the cytoplasmic pools of dopamine.

132.9 OCTOPAMINE RELEASE FROM LIMULUS RETINAL EFFERENTS WITH DEPOLARIZATION INDUCED BY POTASSIUM AND VERATRIDINE. B-A.Battelle. National Eye Institute, NIH, Bethesda, MD 20205

When ventral and lateral eyes of Limulus are incubated in ^{3}H-tyramine, radioactive octopamine and other tyramine metabolites are synthesized and stored exclusively within retinal efferent fibers. Octopamine may be a neurotransmitter of efferent fibers in this visual system responsible for many of the known effects of efferent innervation on lateral eye sensitivity. I have tested whether octopamine can be released from efferent fibers with depolarization and whether octopamine is released selectively or together with other tyramine metabolites.

Ventral and lateral eyes were incubated overnight in ^{3}H-tyramine then rinsed in saline(200µl) that was changed at 5 min intervals. After the rate of efflux of radioactivity stabilized in normal saline, preparations were depolarized with saline containing either a high concentration of K^+(200mM) or veratridine(100µM). Depolarizing ventral and lateral eyes in high K^+ caused transient increases in release of radioactivity from the preparations. Of the total radioactivity released, 70% was collected during the first 5 min. Addition of 40mM Co^{++} to the saline inhibited K^+-stimulated release indicating this release is dependent on the influx of extracellular Ca^{++}. Examination by high voltage electrophoresis of the radioactive substances released revealed that no octopamine was released into normal saline and that octopamine comprised approximately 70-80% of the radioactive substances released in response to K^+ depolarization.

Different results were obtained when veratridine was the depolarizing stimulus. A 10 min exposure of tissues to veratridine caused an elevation in the rate of efflux of radioactivity which increased for 20 min following the initial exposure to the drug. This release was blocked by addition of TTX or Co^{++} or the removal of Na^+ from the bathing saline. Octopamine comprised 70 to 80% of the radioactive substances released during the fist 5 min of depolarization. However by 15 min octopamine was no longer being released even though a significant amount of radioactive octopamine remained in the tissue. The prolonged, elevated release of radioactivity following veratridine- induced depolarization is accounted for entirely by the release of tyramine metabolites other than octopamine.

These results show that octopamine and the other tyramine metabolites are stored in and released from different compartments. The rapid Ca^{++}-dependent release of octopamine is consistent with its proposed role as a neurotramsmitter of efferent fibers. The delayed release of other tyramine metabolites following veratridine depolarization may indicate the presence of a second pool of substances in efferent fibers that is released only after prolonged and extensive depolarization.

132.10 SEROTONIN IS STORED AS A COMPLEX WITH SEROTONIN BINDING PROTEIN IN SITU. K.P. Liu*, H. Tamir, S.E. Karpiak, and M.D. Gershon, N.Y. State Psych. Inst. and Depts. of Psychiatry and Anatomy and Cell Biology, Columbia Univ. Coll. of P&S, New York, NY 10032.

Serotonin binding protein (SBP) is a soluble protein found in synaptic vesicles. Several properties of SBP suit it to serve as a storage protein for the transmitter. It is found in serotonergic neurons of the central (CNS) and enteric (ENS) nervous systems as well as in the neural-crest derived parafollicular cells of the thyroid gland. SBP binds serotonin (5-HT) with very high affinity under conditions (K^+-containing media) that approximate the intracellular milieu; however, the complex is dissociated when it is exposed to extracellular concentrations of Na^+ or Ca^{+2}. We have now obtained evidence that 5-HT is stored as a complex with SBP in situ. Rats, pretreated with clorgyline (25 mg/kg) were perfused intraventricularly (3h) with ^{3}H-5-HT (0.5ml; lmc/ml; 12c/mmole) under light ether anesthesia. Strips of rabbit ENS were incubated with ^{3}H-5-HT (0.10uM; 1 hr) in the presence of clorgyline, pargyline (0.1mM), and desipramine (0.01uM). The tissues were then homogenized in hypotonic KPO_4 buffer; protein-bound ^{3}H-5-HT was obtained from the 100,000g supernatant by filtration on Sephadex G-50 and subjected to SDS-PAGE. Protein bound radioactivity was found to be > 80% non-metabolized ^{3}H-5-HT. Binding to protein of ^{3}H-5-HT added just prior to homogenization was less than 15% of that in situ. The protein-^{3}H-5-HT complexes from brain and gut migrated on the gels with apparent molecular weights of 45K and 56K, corresponding to those measured by SDS-PAGE for purified SBP; however, the 45K molecule predominates when the SBP complex is formed in situ, whereas the 56K molecule predominates when the SBP-^{3}H-5-HT complex is formed in vitro. It is possible that the 56K SBP is characteristic of the molecule in perikarya or non-terminal axons while the 45K molecule is characteristic of terminal varicosities because radioautographic results show that in both CNS and ENS ^{3}H-5-HT is mostly concentrated in terminals. A precursor-product relationship appears to exist between 56K and 45K SBP. The concentration of 5-HT in organotypic tissue cultures with known number of serotonergic neurons was measured and the calculated concentration was found to be >7mM/cell. The binding of 5-HT to SBP within vesicles is therefore important to reduce the osmotic pressure that would build up in synaptic vesicles if 5-HT was free in solution. Supported by NSF grant 09335 and NIH grant NS12969.

132.11 ASSESSMENT OF BLOOD PLATELETS AS A MODEL FOR CNS STUDIES: COMPARATIVE EFFECTS OF CAFFEINE ON 5-HT UPTAKE AND RELEASE MECHANISMS IN RAT PLATELETS AND BRAIN SEROTONIN NEURONS. Dorothy T. Chou, Holly Cuzzone* and Kenneth Hirsh. General Foods Corp. Tech. Ctr., Cranbury, NJ 08512

We have previously reported (1) that caffeine significantly enhanced 5-HT uptake and reduced 5-HT release from crude synaptosomal fractions obtained from rat cerebral cortex and from midbrain raphe region. Blood platelets, as reported by many laboratories and also demonstrated in our own labs, have a very active mechanism for 5-HT uptake and storage. In this regard platelets bear a high degree of similarity to brain serotonin neurons. The present experiments were, therefore, carried out to investigate the effects of caffeine on 5-HT uptake and release from rat platelets in an attempt to assess the possibility of using platelets as a model for studying the CNS effects of caffeine. Platelet rich plasma was prepared from the trunk blood of decapitated rats. Effects of caffeine were investigated at 10^{-7}, 10^{-6}, 10^{-5} & 10^{-4}M (the same concentrations as used in our previous study (1) on brain serotonin neurons) on both the high affinity ^{3}H-5-HT uptake and spontaneous 5-HT release from ^{3}H-5-HT preloaded platelets. The results show that caffeine did not change 5-HT uptake into platelets. In brain synaptosome (1) caffeine increased 5-HT uptake dose-dependently. The present results also revealed that caffeine increased 5-HT release from rat platelets in a concentration-dependent manner. The concentrations 10^{-6}, 10^{-5} & 10^{-4}M increased release significantly compared to control. This finding is also in contrast to that observed in brain serotonin neurons (1) where caffeine decreased 5-HT release. It is concluded, therefore, that the rat blood platelet model is not suitable for studying these CNS actions of caffeine. Furthermore, our observations imply that rat platelet serotonin uptake and release mechanisms are not identical to those mechanisms in brain serotonin neurons.

1. Cuzzone H. et al Fed. Proc. 40(3): 266, 1981

132.12 EFFECTS OF A THIOREACTIVE AGENT, DIAMIDE, ON RABBIT SYNAPTOSOMAL GABA UPTAKE. J.S. Colton*, C.R. Walker. Depts. of Physiology and Internal Medicine, Univ. of Nevada, Reno, Nevada 89557.

Disulfide bonding and sulfhydryl interactions play an important role in the function and modulation of many cellular proteins. The sodium-dependent GABA uptake symport has been shown to be one such sulfhydryl modulated system. Diamide, a thiol oxidizing agent, inhibits GABA uptake by rabbit whole brain synaptosomes in a concentration dependent manner. Our preliminary experiments suggest that diamide inhibits GABA uptake in a complex manner by (1) altering the membrane sodium gradient via inhibition of Na^+, K^+ ATPase, (2) decreasing the availability of synaptosomal ATP, and (3) altering the function of the GABA transport protein(s).

Diamide irreversibly inhibits ^{3}H-GABA uptake with an IC_{50} of $5x10^{-4}$M. Although the uptake affinity for GABA (5µM) is unchanged by 10^{-4}M diamide, the V_{max} is reduced from 3.05 nmoles/mg protein to 2.16 nmoles/mg protein as determined by a Lineweaver-Burk analysis. Diamide at 10^{-3}M depresses V_{max} and causes a decrease in uptake affinity to about 40 µM.

In this study, diamide has been shown to inhibit the Na^+, K^+ ATPase of rabbit synaptosomes. Thus, the effect of altering the Na ion gradient on GABA uptake was investigated. If synaptosomes were preincubated in a Na^+ free media, inhibition of the Na^+, K^+ ATPase would have a minimal effect on the Na^+ gradient when Na^+ was re-introduced for 30 to 60 seconds during the determination of GABA uptake. Therefore, GABA uptake in synaptosomes was determined after a 30 minute preincubation with the drug in the presence or absence of Na ion. In addition, diamide was compared with ouabain, another drug that inhibits both GABA uptake and the Na^+, K^+ ATPase. Controls preincubated in either Na^+ free or Na^+ containing media demonstrated no difference in GABA uptake. Ouabain, 10^{-3}M, inhibited GABA uptake by 10% after Na^+ free preincubation compared with 74% after preincubation in Na^+ containing media. Diamide, 10^{-3}M, inhibited GABA uptake 61% after preincubation in Na^+ free media and 85% after preincubation in Na^+ containing media. Ouabain has also been reported to decrease intracellular ATP levels and may effect GABA uptake in this way when preincubated with synaptosomes in Na^+ free media. Since diamide inhibits GABA uptake when preincubated in a Na^+ free media by about 60%, this suggests a possible involvement in intracellular ATP levels. Preincubation of synaptosomes with diamide at 4°C reduced the inhibition of GABA uptake, but there was still approximately 30% inhibition when compared to controls. This suggests some interaction with the transport protein(s).

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132.13 CLEAVAGE OF PEPTIDES BY PURIFIED RAT BRAIN DIAMINOPEPTIDASE-II. Leonard Sachs* and Neville Marks, Center for Neurochemistry, Rockland Research Institute, Wards Island, N. Y., N. Y. 10035.

Recent data imply that CNS diaminopeptidase-II (DAP-II) is involved in the inactivation of neuropeptides such as enkephalins. To examine this aspect with a purified system we have purified DAPII from rat brain nuclear and mitochondrial-lysosomal fractions (P_1, P_2) utilizing Lys-Ala-2NA incubated at pH 5.5 by detergent extraction, ion-exchange and gel filtration. Purified enzyme gave an apparent M_r of 142 x 10^3 by gel-filtration and was devoid of known exo- and endopeptidase contaminants. In addition to Lys-Ala-2NA, enzyme hydrolyzed Leu-Ala-, Ser-Met, and Ala-Ala-2NA yielding K_{cat}/K_m ratios for all four substrates of 906, 1207, 271 and 207 min^{-1} mM^{-1}, respectively. With respect to other substrates, purified enzyme hydrolyzed tripeptides such as Leu_3, Ala_3, Leu-Gly-Leu, Met-Leu-Tyr, and Ala-Pro-Gly but not Ala-, Leu-, Arg-, or Gly-Gly-Gly. The presence of methyl ester on the C-terminal, or D-residues in positions 2 or 3 of Ala_3 reduced or blocked hydrolysis. No cleavage was observed for enkephalin or Substance P, indicating that this DAP enzyme is unlikely to act as an enkephalinase or be involved in Substance P inactivation. Release of Tyr-Gly from enkephalins therefore must be attributed to another DAP enzyme. Brain homogenates contained in addition to DAP II, enzymes degrading Gly-Arg-2NA (DAP-I) at pH 5.5, and Arg-Arg-2NA (DAP III) at pH 8.0. Detergent extracts of rat brain membranes incubated in the presence of -SH agents and Cl^-, conditions optimal for DAP I, released Tyr-Gly from Met-enkephalin, supporting the notion that this enzyme and not DAP II accounts for enkephalin breakdown. Supported in part by grant NS-12578.

132.14 RELEASE OF MET-ENKEPHALIN-ARG^6-PHE^7 FROM RAT STRIATAL SLICES G. Patey*, M. Chaminade*, P.E. Chabrier* and J.P. Rossier Lab. de Physiologie Nerveuse, C.N.R.S., Gif-sur-Yvette 91190, FRANCE

The common precursor of Leu- and Met-enkephalin in the bovine adrenal medulla has been shown to contain in addition to these opioid peptides the heptapeptide Met-enkephalin-Arg^6-Phe^7 and the octopeptide Met-enkephalin-Arg^6-Gly^7-Leu^8. Both hepta- and octopeptides display potent opioid activity in bioassays. Furthermore the heptapeptide is also present in high amounts in rat striatum, but it is not yet known whether these putative neurotransmitters are released from nerve terminals in the central nervous system.

We present evidence indicating that the heptapeptide is released from rat stiatal slices following a depolarizing stimulus. The experimental procedure has been described (Science, 212:1153, 1981). The amount of heptapeptide was measured in the incubation media and in the slices by radioimmunoassay either directly using an antiserum directed against the heptapeptide or after an HPLC run and treatment with trypsin and carboxypeptidase B using an antiserum directed against Met-enkephalin.

Under these conditions we have shown that following a depolarizing stimulus (50mEq K^+ in the medium) the amount of heptapeptide in the slices is much lower when the stimulus has been done in the presence of calcium ($(CaCl_2)$=2.5mM) than in its absence (3.2+0.4 nmoles/g protein vs 5.0+0.3 nmoles/g protein). However in the absence of any peptidase inhibitor the amount of heptapeptide recovered in the medium after a depolarizing stimulus was not different of that recovered under basal conditions. We then tested the protective effects on released heptapeptide of selective inhibitors of peptidases possibly involved in the catabolism of the enkephalins and the heptapeptide. Thiorphan (0.1uM) selectively inhibits the activity of enkephalin dipeptidyl carboxypeptidase ("enkephalinase") whereas captopril (1uM) inhibits angiotensin converting enzyme (A.C.E.) and bestatin (20uM) inhibits aminopeptidase(s). The simultaneous addition of these inhibitors to the incubation medium greatly enhanced the amount of heptapeptide recovered (0.45+0.10 nmoles/g protein vs 0.09+0.03 nmoles/g protein). In contrast, thiorphan alone was unable to produce such an increase in the amount of heptapeptide recovered (0.04+0.02 nmoles/g protein vs 0.03+0.01 nmoles/g protein), whereas it protected very efficiently Met- and Leu-enkephalin released in the same experiment.

In conclusion we have demonstrated a Ca^{2+}-dependent K^+-evoked release of the heptapeptide Met-enkephalin-Arg^6-Phe^7 from rat striatal slices. This heptapeptide is then rapidly cleaved by peptidases and the enkephalinase is not involved in this enzymatic breakdown.

132.15 ACETYLCHOLINE AND 5-HYDROXYTRYPTAMINE RELEASE ATP FROM ISOLATED MYENTERIC VARICOSITIES. T.D. White and M. Al-Hummayyd*. Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

It has been suggested that ATP might be a non-adrenergic inhibitory neurotransmitter in vertebrate intestinal smooth muscle. It is also possible that ATP is released from noradrenergic nerves to function as a cotransmitter or neuromodulator in the myenteric plexus. We have previously shown release of ATP from isolated myenteric varicosities when they were depolarized with K^+ or veratridine. In the present study, a P_2 preparation of myenteric varicosities was isolated from the small intestines of 3-4 guinea pigs and the effects of acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) on release of ATP directly monitored using firefly luciferin-luciferase in the incubation medium. ACh and nicotine released ATP from myenteric varicosities, whereas the muscarinic agonist, bethanechol, did not. The presence of bethanechol had no effect on subsequent release of ATP by ACh. Release by ACh was Ca^{2+}-dependent and was antagonized competitively by d-tubocurarine. 5-HT also released ATP from myenteric varicosities by a Ca^{2+}-dependent mechanism. Release was more prolonged than that observed with ACh and showed desensitization at higher concentrations of 5-HT. The agonist, 5-methoxytryptamine, did not release ATP but the partial agonist, quipazine, evoked a small release of ATP and antagonized subsequent 5-HT-induced release. Metergoline inhibited the 5-HT-induced release of ATP whereas morphine, phenoxybenzamine and spiroperidol were without effect. Finally, pretreatment of guinea pigs with 6-hydroxydopamine to destroy noradrenergic myenteric varicosities reduced the noradrenaline content of the P_2 preparation by 87% and the ACh and 5-HT induced release of ATP by 48% and 44% respectively. These findings indicate the presence of 5-HT and nicotinic (but not muscarinic) receptors on certain isolated myenteric varicosities, which, when stimulated, elicit the release of ATP from these varicosities. Some, but possibly not all, of the released ATP appears to originate from noradrenergic varicosities present in the preparation.
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132.16 DETERMINATION OF S-ADENOSYLMETHIONINE AND S-ADENOSYLHOMOCYSTEINE IN RAT BRAIN BY HPLC WITH UV DETECTION. Kenneth W. Perry* and Ray W. Fuller. (SPON: Mark M. Foreman) Lilly Research Labs., Eli Lilly and Company, Indianapolis, IN 46285.

S-Adenosylmethionine (SAMe) is the methyl donor in many biochemical transmethylation reactions. S-Adenosylhomocysteine (SAH) is an inhibitory product, and as a consequence the ratio of SAH/SAMe may be an important factor in regulating transmethylation rates. In order to study the effect of altered SAH/SAMe ratios on the N-methylation of norepinephrine to epinephrine in rat brain, we developed a simple, rapid and sensitive assay for SAMe and SAH. The method involves (1) homogenization of brain tissue in 0.1 N TCA, (2) preliminary cleanup of SAMe and SAH on small SP-Sephadex columns, and (3) HPLC on a reverse phase column with absorbance detection at 260 nm. One ml of the supernatant fraction from the TCA homogenate (10 %/o w/v) is placed on the Sephadex SP column (5 x 20 mm bed). The column is washed with 2 ml of 0.05 M ammonium formate pH 2.8, then with 0.5 ml 0.2 M ammonium formate pH 5, and then SAMe and SAH are eluted together with 2 ml of the 0.2 M ammonium formate. This fraction can be evaporated and taken up in a small volume of 0.1 N formic acid before injection onto the reverse phase column if the amount of brain tissue assayed is less than 100 mg. The reverse phase column was a 5 μ C_{18} column used in an ion-pairing mode. Column conditions were: mobile phase 0.05 M sodium acetate pH 5, 6%/o methanol, 20 mg/l sodium octyl sulfonate; temperature 50%; flow rate 1.0 ml/min. The ion-pairing agent is necessary for adequate retention of SAMe. Total run time per sample on the analytical column is 11 min. The overall recovery of SAMe is about 70%/o and of SAH about 60%/o. SAMe levels in hypothalamus and striatum were about equal (26 nmoles/g), but SAH was higher in striatum (2.94 vs. 1.31 nmoles/g). SAMe and SAH in other brain areas were slightly lower than in hypothalamus. After L-dopa (200 mg/kg i.p.) injection into rats, SAMe decreased by 50%/o and SAH increased by 100%/o in hypothalamus, and epinephrine concentration decreased by 20-50%/o. Similar changes occurred after lower doses of L-dopa (10-50 mg/kg) given with carbidopa. Using a radio-enzymatic method, Taylor and Randall found that single or repeated doses of desipramine and other antidepressant drugs decreased SAMe in mouse brain (J. Pharmacol. Exp. Ther. 194, 303, 1975). With our method, we found little or no effect of desipramine on brain levels of SAMe or SAH in either rats or mice. The assay method described here allows direct measurement of SAMe and SAH, is convenient, fast and sensitive, and should be useful in further studies on the influence of the SAH/SAMe ratio on biological transmethylation.

133.1 A CABLE MODEL OF AXO-SPINOUS SYNAPTIC ACTIVATION OF THE SPINY PROJECTION NEURON OF MAMMALIAN NEOSTRIATUM. C. J. Wilson. Dept. Anatomy, Michigan State University, East Lansing, MI 48824.

Quantitative morphological observations on the sizes and shapes of dendritic spines of spiny neostriatal projection neurons have revealed a wide range of variation among these postsynaptic structures. Variation among dendritic spines in spine head diameter, stalk length, stalk diameter and dendritic diameter has been presumed to have effects on the efficacy of synaptic transmission. Quantitative predictions of these effects are presented here based on the assumption of passive linear dendritic membrane, direct measurements of geometrical parameters, and estimates of electrical membrane properties derived from measurements of whole neuron input resistance and time constant. An analytical model of the dendritic spine, based on the treatment presented by Jack, Noble and Tsien (Electric Current Flow in Excitable Cells, Oxford Univ. Press, 1975), yielded impulse responses in the form of infinite series of parabolic cylinder functions. Impulse responses were obtained for the voltage at spine head and base. Simulated responses to time-varying synaptic conductance changes on the spine head were obtained numerically from the impulse responses.

Simulated dendritic spines in the naturally-occurring range of sizes and shapes had very high input impedances which resulted in the generation of giant synaptic potentials, even when synaptic conductances were restricted to a very low (0.1-10 nS) range. Thus synaptic currents were very non-ohmic with conductance and synapses could not be approximated by time varying currents injected at the spine heads. Virtually all the synaptic current flowed into the dendrite, and virtually none outward across the spine membrane. Thus the primary effect of the spine was to limit synaptic current by combination of their large input impedance and the non-linearity of synaptic current flow as postsynaptic potentials approach their reversal potential. Because dendritic spines appeared to be normally operating near this saturation limit even with small synaptic conductance changes, simulated axo-spinous synaptic currents were relatively insensitive to changes in the magnitude of synaptic conductance. The input impedance of spines was very sensitive to the frequency composition of the input waveform. Thus synaptic currents were very much affected by duration of synaptic action. Variation of spine head diameter over the natural range had little affect on axospinous synaptic transmission. Spine stalk diameter and length both appeared to be of great importance, while dendritic input impedance had a strong influence on synaptic currents produced by prolonged, and a smaller effect following transient, conductance changes on the spine head. (Supported by NIH Grant NS 17294).

133.2 OPTICAL RECORDINGS FROM NEURONAL PROCESSES AND THEIR VISUALIZATION BY IONTOPHORETIC INJECTION OF NEW FLUORESCENT VOLTAGE-SENSITIVE DYES. A. Grinvald, R. Hildesheim*, A. Agmon* and A. Fine* Dept. of Neurobiology, Weizmann Institute of Science, Rehovot, Israel.

Injection of fluorescent voltage-sensitive dyes into a neuron is the method of choice for optical recording of electrical activity and synaptic responses from the processes of single nerve cells in the CNS. To achieve this goal we designed and synthesized new probes for an iontophoretic injection, attempting to optimize the following dye properties: the probes should bind specifically and with high fluorescence to the cell membrane, be nonfluorescent in water, be easily ejectable through microelectrodes, quickly diffuse within the fine processes, not leak from the cell, cause minimal pharmacological side effects, cause negligible photodynamic damage, have photochemical stability, and obviously show high degree of voltage sensitivity. To select the best probe we injected these dyes into neuroblastoma cells in culture and into leech sensory cells in isolated segmental ganglia. Three styryl dyes, RH-292, RH-355 and RH-425, having a double-positive charge, proved to be suitable in these experiments. (RH-292 is: 4-[4'p-dibutyl-amino-phenyl-1':3'-dienyl] 1-γ-triethylammonium-propyl pyridinium bromide; RH-425 is a trimethylammonium analog. RH-355 is similar to RH-425 but has a p-dimethyl aminophenyl group.) Thin-wall microelectrodes were filled with 0.1M solution of the dye dissolved in distilled water. The iontophoretic injection and the intracellular recordings could be done with the same electrode having a resistance of 80-160MΩ (20MΩ resistance if filled with 3M KCl). The injected dye diffused through the processes of neuroblastoma and did not have any pronounced adverse effect on cell viability or electrical activity. RH-355 diffused quickly in the arborization of leech sensory neurons, and in less than 30 minutes stained fine branches within the neuropile, which could be visualized in unfixed live ganglia. Thus, these red-fluorescing dyes may be useful in double-label experiments together with Lucifer Yellow. Using a DC-operated lamp (HBO/100W) and an optical recording system (Science 212, 1164 (1981)), changes in membrane potential in a section of a main process could be monitored optically, from processes of injected neurons. The signal-to-RMS-noise ratio was ∿9-12 when 50 trials were averaged. Theoretical analysis predicts a considerable improvement in the signal-to-noise ratio with modified apparatus and/or better optical probes. These improvements will be discussed.

Supported by a grant from the TRF.

133.3 IN VIVO STIMULATION (10Hz) OF THE EDINGER-WESTPHAL NUCLEUS PRODUCES AN INCREASED NUMERICAL DENSITY OF CLEAR VESICLES AND OF DENSE CORE VESICLES IN THE PRESYNAPTIC TERMINAL OF THE CHICK CILIARY GANGLION. E. PHILIPPE and J.P. TREMBLAY (SPON: L.Poirier) Lab. de Neurobiologie, Dépt. d'Anatomie, Université Laval, Québec, Canada.

Recent studies (Tremblay and Philippe, Exp. Brain Res. 43, 439, 1981; Philippe and Tremblay, Neurosc. Lett. 24, 307, 1981) have shown that stimulation [100Hz, 15 min.] of the Edinger-Westphal nucleus (EWn) induces a decreased numerical density of clear and dense core vesicles and an increased numerical density of coated vesicles in the cholinergic presynaptic terminal of the chick ciliary ganglion. This study analyses the effects of stimulation at a lower frequency. The EWn of one day old chicks was stimulated in vivo during 15 min at 10 Hz with a monopolar electrode placed stereostatically. The ciliary ganglion was fixed by injecting 2% formaldehyde and 1% glutaraldehyde in cacodylate buffer 0.12M, pH 7.4, in the orbital cavity 2 min before the end of the stimulation. The ganglion was then dissected out and placed overnight in the same fixative at 4°C. Eighty microns thick longitudinal slices of the ganglion were then obtained with a Smith Farquhar tissue shopper. After rincing in cacodylate (10 hours), the tissue was postfixed in 1% O_sO_4, dehydrated in alcool, stained in bloc with uranyl acetate, passed briefly in pronylene oxide and embedded in epon. Ultrathin sections were collected on a 150 mesh copper grid coated with formvar. Eight (4 controls, 4 stimulated) longitudinal calyx sections were completely reconstructed using about 40 overlapping pictures for each. The ciliary cells whose calyx was reconstructed had to be located in a square of the copper grid completely filled with epon or formvar to prevent any density modification due to shrinking of the section. An excentric nucleus had to be present to insure that the section was from the central portion of the ciliary cell. This experiment shows that a stimulation at low frequency [10 Hz, 15 min.] induces an increase of the numerical density of the clear synaptic vesicles [controls: 88.6 ± 25.3/um^2; stimulated: 134.5 ± 27.6/um^2] and the dense core vesicles [controls: 0.98 ± 0.16/um^2, stimulated: 2.52 ± 0.62/um^2]. No change was observed in the numerical density of the coated vesicles [controls: 0.90 ± 0.30/um^2; stimulated: 1.12 ± 0.16/um^2]. These differences were found significant (p 0.05) using a Mann-Whitney U test. These results can be attributed to either the formation in the nerve ending of new vesicles from precursor membrane or to anterograde axonal transport. These results suggest that at low stimulation rate the formation or transport of new vesicles exceed the reduction due to exocytosis.

133.4 SYNAPTIC BOUTONS PROXIMAL TO THE AFFERENT AXON ARE MORE ACTIVE THAN DISTAL BOUTONS IN ADULT CHICK CILIARY GANGLION. R.E. Laurie* and J.P. Tremblay (Spon. R. Boucher) Dept. Anatomy, Laval Univ., P.Q., Can. G1K 7P4.

Synaptic boutons on a particular ciliary cell arise from a common axon originating in the Edinger-Westphal nucleus (EWn). This afferent axon arrives on the ciliary cell at the pole at which the efferent axon is present (axonal pole). It forms several synaptic boutons in this area and fewer boutons at the opposite pole of the ciliary cell (nuclear pole). The individual participation in chemical transmission of distinct boutons can be investigated by quantifying morphological changes produced by repetitive stimulation of the EWn.

Four thirteen week old roosters were anesthetized with either methoxyflurane or halothane. Stimulation at 100 Hz of the left EWn was carried out for 15 min. Four control animals were anesthetized but not stimulated. A mixed aldehyde fixative was injected in the orbital cavity during the last 2.5 min of stimulation. The left ciliary ganglion was prepared for EM using conventional techniques. The observed ciliary cells were divided into 3 regions of equal size (axonal and nuclear poles and the central zone). For each animal five synaptic boutons were photographed at the axonal pole and five at the nuclear pole. A maximum of one bouton at the axonal pole and one bouton at the nuclear pole were selected from a given ciliary neuron. Bouton selection and measurements were done as a blind study. For boutons at the axonal pole the numerical density on area (number/μm^2) of clear and dense core vesicles was reduced following stimulation (91.149 ± 7.900 vs 59.841 ± 8.760 for clear vesicles, 1.160 ± 0.155 vs 0.476 ± 0.104 for dense core vesicles). An increased surface density of vacuoles ($\mu m^2/\mu m^3$) as well as bouton perimeter (μm) were also noted in the axonal pole boutons following stimulation (0.530 ± 0.066 vs 1.018 ± 0.185 for vacuoles, 10.639 ± 1.042 vs 14.360 ± 1.424 for bouton perimeter). Values given are means ± standard error of means for control vs stimulated boutons ($p < 0.05$). These characteristic morphological changes were not observed in the boutons located at the nuclear pole. This suggests that boutons located proximal to the afferent axon (at the axonal pole) participate more in chemical transmission than those located distally (at the nuclear pole).

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133.5 MODIFIED SYNAPSES IN THE VENTRAL HORN OF PARAPLEGIC MONKEYS. T. Khan and G. Gaik. RER&D Center, VAMC, Hines, IL 60141 and Loyola University School of Dentistry, Maywood, IL 60153.

Electrophysiological studies have demonstrated enlarged excitatory postsynaptic potentials below the level of spinal cord injury. The primary purpose of this study was to investigate the effects of spinal cord injury on synaptic morphology in the ventral horn below the lesion.

The monkey spinal cords were contused by dropping a 30 gm weight from a height of 22cm at the T11 level. The animals remained completely paraplegic for the duration of the study. At different time intervals, they were anesthetized with sodium pentabarbitol, and perfused with Karnovsky's fixative. Pieces of the spinal cord 1-2cm below the lesion were washed in cacodylate buffer and post-fixed in osmium tetroxide for electron microscopy.

In the control spinal cords, the various synaptic types were the same as those described by others in the ventral horn.

Twenty-four hours after the injury, the major change was the almost complete absence of the F-type synapse. The S-type synapses showed a variety of vesicular changes. In some the vesicles were clustered, while in others they were densely packed. In most instances, the S-type demonstrated a paucity of vesicles. Accumulation of glycogen within the synapses was a frequent observation. Higher vesicle packing density than normal and the absence of associated stacked layers of rough endoplasmic reticulum were noted in the C-type synapses.

Thirteen weeks post-injury, the F-type synapses were identifiable again even though they were not as numerous as in the controls. Although both S- and F-types had normal active zone attachments, the remainder of the junctional interface was dissociated. This phenomenon of dissociation was a prominent characteristic at this stage.

At thirty weeks post-trauma the mitochondria within all synaptic types were swollen with few internal cristae. The active zones were normal and junctional interface dissociation was absent. There were few vesicles within most of the synaptic types and those present were clumped near the active zone. There was no glycogen accumulation observed within the synapes.

Quantitation of the different synaptic types described at various time intervals after the injury is underway and will be presented in conjunction with the morphological data.

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133.6 SYNAPTIC APPOSITION, GLIAL OBSTRUCTION, AND SYNAPTIC STRENGTH AT FROG NEUROMUSCULAR JUNCTIONS. A.A. Herrera and A.D. Grinnell, Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA, 90024. AAH now at Dept. Biological Sciences, University of Southern California, Los Angeles, CA 90007.

Our previous work has shown that unilateral denervation of the sartorius muscle in Rana pipiens causes a 3 to 8 fold increase in transmitter release in the contralateral sartorius, without an increase in synaptic size as seen in the light microscope. We have looked for ultrastructural correlates of differences in release. Using intracellular recording, quantal content was measured at 5 endplates on adjacent muscle fibers in a contralateral sartorius in 0.3 mM Ca^{2+} Ringer. These neuromuscular junctions were marked, stained, measured and drawn with the light microscope, and embedded in Epon. Thin sections were taken every 4 μm for approximately 300 μm, yielding between 52 and 94 cross-sectional profiles of terminal branches for each of the 5 junctions. Morphological measurements were correlated with total transmitter release and release per unit nerve terminal length for each junction, which we take to be a useful measure of the inherent transmitter release capability of a given terminal. There was no correlation between release per unit length and number of mitochondria, density of synaptic vesicles within 0.2 μm of the presynaptic membrane, the incidence of dense-cored vesicles, or an index of cross-sectional shape measuring "flatness" of the terminal profile. Weakly positive, but non-significant correlations existed between release per unit length and the perimeter or cross-sectional area of terminal profiles. However, strong positive correlations were seen between release per unit length and 1) mean cross-sectional width of presynaptic membrane in close apposition to postsynaptic membrane (apposition width) or 2) the proportion of the terminal membrane in synaptic contact with the postsynaptic membrane (apposition width/terminal perimeter). A strong positive correlation was also seen between an estimate of the total area of synaptic apposition (apposition width x total length of terminal branches) and total transmitter release.

These observations suggest that obstruction of synaptic clefts by Schwann cells may be an important modulator of release efficacy. On the other hand, if active zone length is directly proportional to apposition width, the differences in apposition width and area of synaptic contact would not account quantitatively for the differences in release seen, unless one assumes a highly non-linear relationship between active zone length and release. The morphological changes may therefore be a consequence, rather than a cause, of enhanced release. Alternatively, the observed changes may be only part of an ensemble of changes that, in total, are responsible for differences in release.

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133.7 THE SYNAPTIC BOUTON OF MAMMALIAN SPINAL CORD NEURONS RELEASES ONE OR LESS EXCITATORY TRANSMITTER QUANTA. P. Nelson, R. Macdonald, E. Neale, R. Pun, K. Marshall, C. Christian and W. Sheriff*. Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD.

We have studied excitatory synaptic inputs to mouse spinal neurons with intracellular microelectrodes in a dissociated cell culture system. EPSPs were elicited by stimulating either other spinal cord (SC) neurons (SC-SC connections) or co-cultured dorsal root ganglion (DRG) neurons (DRG-SC connections). Computer analysis of a series of evoked synaptic potentials provided the mean and variance of the EPSPs in the series. The coefficient of variation (C.V.) and hence the mean quantal content, m, of the series ($m=1/C.V.^2$) could be derived according to the assumptions of a Poisson release process. When release was varied over a wide range by alterations in stimulation rate or in external Ca^{++} concentration, it was evident that the release process was better modeled by binomial than by Poisson statistics. If quantal amplitude can be assumed to be constant under a variety of conditions which affect the presynaptic release mechanism, the probability of release, p, and the number of release elements, n, can be calculated. (Correction for non-linear summation must be made where appropriate). The probability of release may be quite high, 0.7 or even higher, particularly with the SC-SC connections. For DRG-SC connection, p was somewhat lower, about 0.35.

Horse radish peroxidase injection of presynaptic neurons allows visualization of their boutons so that the number of boutons subserving a physiologically analyzed excitatory connection can be determined. We find that the number of boutons is equal to, or slightly greater than the number of physiologically determined release elements. We would suggest that the statistically defined release element may be identified with the morphological element, the bouton. The probability of release from each release element can vary only between zero and one. Together with the results of others (Korn et al., Science 213, 1981; Jack et al., J. Physiol. 321, 1981), this would indicate that there may be some limiting mechanism within the excitatory bouton which allows no more than one transmitter quantum to be released with each presynaptic action potential.

133.8 EFFECTS OF POSTSYNAPTIC POLARIZATION ON MONOSYNAPTIC EXCITATORY POSTSYNAPTIC POTENTIALS (EPSPs) IN SPINAL CORD CULTURE. R.Y.K. PUN, G.L. WESTBROOK*, P.G. NELSON. Lab. Dev. Neurobiol., NICHD, NIH, Bethesda, MD 20205.

In an accompanying abstract, we have described the statistical nature of release of the monosynaptic EPSPs in mouse spinal cord (SC) cultures (see Nelson et al., these abstracts). We present here results on the reversal potentials of these EPSPs.

Intracellular recordings were obtained from dissociated fetal mouse SC cells 4 - 8 weeks in culture with 3 M KAc electrodes. Following the identification of a monosynaptic EPSP between a dorsal ganglion (DRG) cell and a SC cell, or between two SC cells, the postsynaptic cell was penetrated with a second electrode containing either tetraethylammonium (TEA^+, 0.5M) or caesium (Cs^+, 1M) salt solution. This second electrode was used to inject current and to polarize the membrane potential (MP) of the cell. After injection of either TEA^+ or Cs^+ ions to block the delayed rectification, the effects of steady polarizing currents on the EPSPs were examined.

As expected of a chemical synapse, the amplitude of the EPSP was increased when the MP was hyperpolarized, and was reduced when the MP was depolarized. This was true for both the DRG-SC and SC-SC synapses. In some studies it was difficult to reverse the initial phase of the DRG-SC EPSP. In most cases, polarizing the MP to > +30 mV brought about a reversal. We have also observed sudden shifts of MP to a more depolarized state when near the reversal potential. Furthermore, the reversal potential for the monosynaptic EPSP was more positive than that for the polysynaptic EPSPs on the same cell. These observations were similar to the complex behavior reported for Ia EPSPs in motoneurons (Engberg and Marshall, Neurosci., 4: 1583-1591, 1979). The mean reversal potential for the DRG-SC pair was +11.7 + 1.9mV (mean ± s.e.m., n=5); and +2.7 + 2.5mV (n=15) for the SC-SC pair. These values are significantly different (Wilcoxon 2-Sample Rank, $p<0.02$). As expected, there was a greater scatter in the reversal potentials within the SC pairs. It is possible that the more positive reversal for the DRG-SC cells is related to a more distal location of the synapse; however, morphological data do not support this (Neale et al.; Brain Res., 152: 265-282, 1978; in preparation, 1982). The positive reversal potential suggests that the increase in conductance at the postsynaptic membrane is predominantly to Na^+ ions. The role of Ca^{++} and K^+ ions in generating the EPSP is at present unknown.

Despite the heterogeneity of the cells in culture, the difference in the reversal potential and the release parameters of the DRG-SC and SC-SC connection (see Nelson et al.,) may reflect a dissimilarity in the mechanism of the two classes of synapses.

133.9 UNIFORMITY OF TRANSMITTER RELEASE ALONG THE LENGTH OF FROG MOTOR NERVE TERMINALS. Albert J. D'Alonzo* and Alan D. Grinnell. Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA 90024.

Correlated physiologic and morphologic studies of single identified frog neuromuscular junctions have revealed large differences in quantal content and release per unit terminal length. In order to understand the basis for these differences, and to help in the interpretation of alterations in release efficacy resulting from experimentally-produced changes in motor unit size or contralateral deervation, it is important to know whether the probability of release is the same for all parts of the nerve terminal. Bennett and Lavidis (J. Gen. Physiol. 74:429, 1979) have reported that release in low calcium Ringer can be highly non-uniform. We are studying this problem using the following technique: living neuromuscular junctions from frog Sartorius muscles, stretched to 110% of their resting length, are visualized using an indirect immunologic fluorescent staining procedure involving a monoclonal antibody against the acetylcholine receptor (kindly provided by Dr. J. Lindstrom). This allows two KCl-filled microelectrodes to be placed just beyond the far ends of the junction to be studied. Extracellular calcium concentration is reduced to 0.25mM (magnesium 2.25mM), lowering the quantal content of the end-plate potential (EPP) to 0.5 or lower, so that most responses consist of a single quantum. The ratio of the peak amplitude of each single quantum EPP recorded simultaneously from the two electrodes is used to localize the release site between the two electrodes. Five hundred or more responses are recorded from each terminal. Localization of release is calibrated by iontophoretically applying calcium at known points along the nerve terminal during stimulation. The ratio of peak amplitudes of the calcium-enhanced multiquantal responses are used to correlate ratios and position. After each experiment, muscles are fixed and the nerve terminals stained. Comparing the distribution of amplitude ratios with nerve terminal distribution permits determination of the probability of release for each position along the nerve terminal. Preliminary results indicate that release is uniform along the length of nerve terminal at the low levels of release studied. (Supported by an NRSA training grant #NS 07101 (AJD) and USPHS grant #NS 06232 to ADG).

133.10 THE STRUCTURE AND DISTRIBUTION OF CLATHRIN COATED VESICLES AT NEUROMUSCULAR JUNCTIONS. J.E. Smith, and D.O. Smith. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

Clathrin coated vesicles (CVs) have been proposed as intermediates in recycling of synaptic vesicle membrane (Heuser and Reese, J. Cell Biol., 57: 315, 1973). To complement their study, we chose to examine in further detail the structure and distribution of clathrin coated structures at the frog neuromuscular junction (nmj). Cutaneous-pectoris nerve-muscle preparations in normal Ca^{2+} (2 mM) Ringer's solution at 10°C were stimulated electrically at 10 Hz for 15 min. Subsequently, they were fixed during stimulation with barbital-buffered 2% OsO_4 solution containing elevated Ca^{2+} (20 mM). Control muscles were fixed without any stimulation. In separate experiments, this same procedure was repeated using nonstimulated and stimulated junctions which had been exposed previously to elevated Ca^{2+} for 1 hr at 10°C. All muscles were then stained en bloc in uranyl acetate and prepared routinely for electron microscopy.

Micrographs obtained from serial thin sections of junctions in cross-section were examined for the presence of coated structures: coated pits, CVs adjacent to membrane (either presynaptic or cisternal, but not synaptic vesicle membrane), and isolated CVs located at least 50 nm from presynaptic or cisternal membrane.

There were 5 times more coated structures in stimulated than nonstimulated junctions. This difference is highly significant and is 1.5 times larger than that reported by Heuser and Reese. There was no difference between the total number of coated vesicular structures in junctions exposed to elevated Ca^{2+} and those exposed to normal Ca^{2+}. The greatest number of coated structures at stimulated junctions were CVs directly adjacent to membrane, accounting for 55% of the total coated structures; at nonstimulated junctions, these accounted for only 10% of all coated structures.

The precise site of CVs that appeared to be isolated in single sections was located and examined in adjacent serial sections. Approximately 3% of the total number of CVs appeared truly isolated from any membranous connection. Conversely, at least 51% exhibited a highly probable connection with presynaptic membrane in an adjacent section; they appeared to be coated pits and not isolated vesicles. If CVs do pinch off by endocytosis and shed their clathrin coat to recycle presynaptic membrane, the process must be so rapid as to be practically undetectable.

This work was supported by NIH grants NS13600 and NS00380.

133.11 ATP RELEASE FROM CHOLINERGIC SYNAPSES. E.S. Schweitzer & R.B. Kelly. Dept of Biochem & Biophys, Univ. of Cal., School of Med. San Francisco, California 94143

Cholinergic synaptic vesicles from Torpedo electric organ contain both ACh and ATP. The vesicle fusion model of neurotransmitter release predicts that the contents of the vesicles (ATP as well as ACh) should be released when the synaptic terminals are stimulated. In contrast, this release of ATP would not be expected if ACh were released from the cytoplasm. The amount of ATP released from Torpedo synaptosomes can be continuously monitored by suspending them in physiological saline containing a luciferin-luciferase assay system. When synaptosomes are suspended in such a solution, depolarization with a high K solution causes the release of ATP into the bathing medium. This release is graded as a function of depolarization, as indicated by increasing ATP release with increasing concentrations of K. The release is also Ca dependent, and can be blocked by the addition of excess EGTA. The divalent ions Ni, Co and Cd also inhibit this release.

The possibility that the ATP is released by intra or extra-terminal mitochondria is eliminated by the observation that FCCP, a mitochondrial uncoupler, and atractyloside, which blocks ATP transport by mitochondria, do not block the K-stimulated release.

The release of ATP under conditions expected to stimulate neurotransmitter release suggests that ATP is released concurrently with ACh as a result of neurosecretion. In support of this model, parallel experiments demonstrate that these synaptosomes release both ACh and ATP under depolarizing conditions in the presence of Ca. The relative amounts of ACh and ATP correspond approximately to those found inside synaptic vesicles.

Taken together, this evidence suggests that ATP release provides an accurate picture of neurotransmitter release in vitro, and that the release of neurotransmitter occurs by the fusion of synaptic vesicles with the plasma membrane, resulting in the release of vesicle contents into the extracellular space.

Under these conditions, in which release appears to be mediated by exocytosis, the muscarinic agonists carbachol and oxotremorine have no effect on ATP release. In contrast, treatment with TFP, a drug known to block calmodulin-mediated processes, completely inhibits release of ATP, but does not block Ca influx into the terminals. This result implies that a calmodulin-dependent step is required for intraterminal Ca to trigger neurosecretion.

133.12 BIOGENESIS AND MAINTENANCE OF PRESYNAPTIC MACROMOLECULES. J.A. Garner* and H.R. Mahler. Brain Research Group, Dept. of Chemistry, Indiana University, Bloomington, IN 47405

The biogenesis and maintenance of subcellular compartments in the presynaptic terminals of retinal ganglion neurons was investigated by combining two powerful techniques: axonal transport of radiolabeled subcellular compartments to the terminal regions and subsequent synaptosomal fractionation. The protein constituents of the different subcellular compartments (i.e. membranous elements, cytoplasmic matrix proteins and cytoskeleton) are all synthesized in the neuron cell body concurrently, yet each compartment travels through the axons to the terminals at a different rate. By harvesting at appropriate times after labeling, we can obtain terminal regions that only contain radiolabeled species of single subcellular compartments. In order to differentiate between radiolabeled macromolecules in axons and presynaptic terminals, we have prepared synaptosomes (HS) and synaptosomally-derived fractions [such as synaptic plasma membranes (SPM) and soluble synaptoplasm (SS)] from the radiolabeled terminals.

We have compared two presynaptic subcellular compartments in this study: proteins and glycoproteins of membranous elements supplied by the fast component (FC) of axonal transport, and putative cytoplasmic matrix proteins supplied by slow component b (SCb). Adult guinea pigs (300-400 g) were injected intraocularly with ^{35}S-methionine or a mix of ^{3}H-lysine/^{3}H-proline. A dynamic representation of the movement into the terminal and maintenance of these transport components was obtained by harvesting at many time points during their entrance and persistence in terminal regions. At harvest, HS were prepared according to Carlin *et al.* [J. Cell Biol. 86, 831-843 (1980)], and were osmotically shocked to produce SPM and SS. All fractions were subjected to SDS-PAGE and radioactive species revealed by fluorography. In addition, radioactive species in 1 mm segments of optic nerves and tracts were compared to terminal profiles.

Results obtained lead to the following conclusions: 1) the 10 major FC proteins arrive earlier than 3h and persist for at least 72 h; 2) minor FC species appear earlier and disappear earlier than major proteins; 3) the quantitative distribution of radiolabeled FC proteins in HS is different from that of whole homogenate; 4) several FC proteins are enriched in SPM relative to HS; 5) unlike other systems, no major rapidly turning over FC protein is seen; 6) the SCb proteins appear to enter the terminal together, peak together (with minor exceptions) and start to decline together; 7) the qualitative distribution of radiolabeled SCb proteins, unlike FC, varies little among fractions and 8) a much larger precentage of SCb than FC is found in SS. Points 6)-8) support the inference that SCb may represent the cytoplasmic matrix. (Supported by NS 06810 and NS 08309 from NIH)

133.13 SUPPRESSION OF ABNORMAL SYNAPTIC VESICLE DEPLETION BY DIVALENT CATIONS IN THE DROSOPHILA MUTANT SHIBIRE. W. J. Costello and L. Salkoff, Dept. Zoology/Coll. Osteo. Med., Ohio Univ., Athens, OH 45701 and Biology Dept., Yale Univ., New Haven, CT 06511.

The genetic approach to explain complex problems in synaptic physiology can be undertaken to great advantage in studies using the fruit fly Drosophila melanogaster. One mutant, shibire^{ts1} (shi) is temperature sensitive, causing reversible paralysis of flies @ temperatures above 28°C. Paralysis is due to a blockage of synaptic transmission. Morphological studies have shown that the synapses of heat-pulsed flies contain few or no vesicles (Poodry & Edgar, 1979, J. Cell Biol. 81:520-52). There are numerous large membrane bound cisternae in these depleted synapses.

We have found that this depletion at the synapse is prevented by internal perfusion of flies with salines containing higher than normal concentrations of divalent cations. In low temperature studies, shi flies were held @ 18°C; the flies were dissected at this temperature and prepared with cold fixative. For high temperature studies, shi flies were divided into two groups and treated @ 18°C. One group was injected with saline containing normal Ca^{++} (2mM); individual flies of the second group were pre-treated by injecting with saline containing either 18mM Ca^{++} or 18mM Mg^{++} & 2mM Ca^{++}. This level of Mg^{++} doesn't block transmission. Both groups were then kept at 30°C for 20 min and then fixed. Neuromuscular synapses of DLM in untreated shi flies @ 30°C had few vesicles but many cisternae. Flies pre-treated with Ca^{++} or Mg^{++} and held @ 30°C had normally appearing synapses. Many vesicles but few cisternae were present.

Intracellular records of the dorsal longitudinal muscles (DLM) were also made on parallel sets of flies by stimulating the motor axons. At 18°C, shi flies appeared normal tolerating stimulus frequencies up to 20 Hz. Untreated shi flies @ 30°C showed a decline in size of excitatory junctional potentials (EJP) at stimulus frequencies <1Hz. In shi flies pre-treated with Ca^{++} or Mg^{++}, higher frequencies were tolerated before EJP decline (6-12 Hz).

We also observed that cisterna-like structures were in close association with the sarcolemma in untreated shi flies subjected to 30°C. In flies pre-treated with the cations, the sarcolemma lacked these cisterna-like structures. The evidence suggests that the divalent cations may be mitigating an overall membrane defect which is causing the abnormal temperature-associated phenomena.

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133.14 INCREASED ^{3}H-FUCOSE INCORPORATION FOLLOWING NEURONAL ACTIVITY IN THE RAT SUPERIOR CERVICAL GANGLION. Clark A. Briggs, George C. Stone, Lohri E. Grishow,* Richard Hammerschlag and Donald A. McAfee, Div. Neurosciences, City of Hope Res. Inst., Duarte, CA 91010.

Glycoproteins, embedded in the plasma membrane with their oligosaccharide exposed to the extracellular medium, appear to function as cellular recognition molecules in many systems. A great deal of specificity can be encoded into the oligosaccharide chain and it is conceivable that glycoproteins could be important sites of modulation for plasticity in synaptic function. Consequently, we have examined the effect of changes in synaptic activity on the glycoprotein metabolism of the ganglion as measured by fucose incorporation.

Homologous pairs of ganglia were preincubated for 2 hr in 0.5 ml of oxygenated Locke's solution (23°C) containing the essential amino acids and then were incubated for 1 hr in fresh Locke's - amino acids containing ^{3}H-fucose (200 uCi, 5 uM) and ^{14}C-leucine (1 uCi, 6 uM). One member of each pair was stimulated at 10 Hz during the middle 20 min of the 1 hr incubation period, while the contralateral ganglion served as the unstimulated control. At the end of the experiment, ganglia were homogenized in 10% TCA or prepared for separation of proteins by SDS-polyacrylamide gel electrophoresis.

Stimulation of the preganglionic nerve increased incorporation of ^{3}H-fucose into glycoprotein to 170% of control (116%-260%, 6 experiments). ^{14}C-leucine incorporation was also increased to 165% of control (150%-175%, 3 experiments). However, increased fucose incorporation did not depend on de novo protein synthesis. Cycloheximide (0.1 mg/ml) reduced ^{14}C-leucine incorporation to negligible levels but preganglionic stimulation still increased ^{3}H-fucose incorporation to 169% of control (160%-186%, 3 experiments). The increase in incorporation depended on postganglionic neuron discharge. Addition of curare plus atropine to block cholinergic transmission also blocked the effect of stimulation on ^{3}H-fucose incorporation (2 experiments). Postganglionic nerve stimulation (2 experiments) increased ^{3}H-fucose and ^{14}C-leucine incorporation to an extent comparable to preganglionic stimulation. Under the above protocol, preganglionic stimulation had no effect on the intracellular pools of TCA-soluble ^{3}H-fucose.

While little is known about glycoprotein function, these experiments indicate that synaptic activity can increase glycoprotein metabolism in the postganglionic neuron. A more difficult problem will be determining the extent to which increased glycoprotein fucosylation occurs in the Golgi complex or the postsynaptic membrane. (Supported by grants NSF BNS 79-12394, NSF BNS 81-12129, NIH NS-12116 and BRSG.)

133.15 COMPARISON OF ZINC AND COPPER IN SUBCELLULAR FRACTIONS OF RAT HIPPOCAMPUS AND CEREBELLUM. D.A. Taylor*, N.F. Harris, and I.L. Crawford. Depts. of Pharmacology and Neurology, Univ. Tex. Hlth. Sci. Ctr. and V.A. Medical Center, Dallas, TX 75235.

Quantitative analyses indicate higher zinc (Zn) concentrations in hippocampus than in other brain regions. Autoradiography and histochemistry suggest the localization of Zn in mossy fiber boutons; supporting quantitative evidence is not available. Therefore, Zn and copper (Cu) concentrations were compared in subcellular fractions of rat hippocampus and cerebellum. Hippocampal and cerebellar homogenates (10% w/v 0.32M sucrose) were spun at 1000 x g to yield a pellet (P1) and a supernatant (S1). From the S1, a crude mitochondrial pellet (P2) was obtained and fractionated over a discontinuous sucrose gradient. Final fractions analyzed for metal were the interfaces between 0.32M and 0.8M sucrose (A2); 0.8M and 1.2M sucrose (B2); the pellet (C2) below 1.2M sucrose. P1 was fractionated with 24% w/v Ficoll in 0.32M sucrose to separate nuclei and cell debris (NP) from a pellet containing a large synaptosomal component and myelin. The resuspended pellet was layered over a discontinuous sucrose gradient; the interfaces at 0.32M/1.0M sucrose (A1) and at 1.0M/1.4M sucrose (B1) were analyzed for metal content. All fractions were analyzed for Zn and Cu by electrothermal atomic absorption spectroscopy. The following mean concentrations of metal were obtained from four assays:

Fraction	Subcellular contents	Hippocampus ng/mg protein		Cerebellum ng/mg protein	
		Zn	Cu	Zn	Cu
H	homogenate	199	47	161	54
P1	crude nuclear pellet	213	72	169	50
S1	microsomes; cytosol	230	35	156	61
P2	crude mitochondrial pellet	196	61	195	106
A2	membrane; myelin	202	nd	130	nd
B2	synaptosomes (0.5 µm)	177	38	170	29
C2	mitochondria	140	71	110	162
NP	nuclei; cell debris	235	161	175	156
A1	myelin fragments	253	nd	182	nd
B1	mossy fiber boutons (5.0 µm)	597	333	667	482

The SEM for Zn values were < 20% of the means; those for Cu were more variable. nd = not detectable.

Zn and Cu content was significantly greater ($p<0.05$) in hippocampal and cerebellar fractions B1 than fractions B2. The content of these metals in B2 was not different from other fractions. Our quantitative evidence supports the suggested localization of Zn in large synaptosomes isolated from hippocampus and cerebellum. The high Cu content in B1 was unexpected and is yet to be explained. (Supported by: NIH NIGMS GM07062 and VA MRIS 1604).

134.1 **INTRACELLULAR CALCIUM DIFFUSION AND TRANSMISSION AT THE FROG NEUROMUSCULAR JUNCTION.** N. Stockbridge* & J.W. Moore. Department of Physiology, Duke University Medical Center, Durham, NC 27710.

The consequences of intracellular calcium binding sites on transmitter release at a synapse have been examined by computer simulation. At the nerve terminal of the frog neuromuscular junction, it predicts (1) a delay, rise, and fall of transmitter release (assumed proportional to $[Ca]_i^4$) similar to the endplate current, and (2) amplitude and time course of facilitation similar to that observed.

Transmembrane influx of calcium has been modeled as either (1) a square wave pulse of calcium, or (2) as a current with the shape described by Llinas, et al. (1981) at the squid giant synapse, but with compression of the time course more appropriate for the frog nerve terminal. The calcium pump has been modelled as (1) a first-order rate process dependent on the submembrane calcium concentration, or (2) as a saturable process similar to that described by Blaustein (1976).

Intracellular calcium is presumed to be rapidly equilibrated with uniformly distributed fixed nonsaturable sites in the cytoplasm. Such binding effectively reduces the rate of diffusion (Hodgkin & Keynes, 1959). An upper limit to the rate of diffusion in squid axoplasm is fixed by Blaustein & Hodgkin (1969) as 6×10^{-6} cm^2/s, in experiments in which millimolar calcium was used. Because it appears that their initial calcium concentration was high enough to saturate the sites and thereby increase the apparent diffusion constant, we use a somewhat lower value ($10^{-6} cm^2/s$) in our model.

The unidimensional model was constructed by dividing the 1 μ radius of the nerve terminal into discrete annuli of 100 Å. The differential equation for diffusion was written in a finite difference form and solved for successive intervals of 10 μs by standard means (Crank & Nicholson, 1949).

Several interesting observations emerge:

(1) Diffusion of calcium is so slow that
  (a) calcium involved in transmitter release would have to enter the terminal very near to release sites,
  (b) the calcium concentration near the membrane remains high for tens of milliseconds, giving the pump an opportunity to extrude a reasonable fraction of the influx, and
  (c) mitochondria and other organelles (usually away from the vesicle region) are unlikely to participate in regulation of $[Ca]_i$ during the period of phasic transmitter release or early facilitation.

(2) Transmitter release turns off with about the time course of the endplate current.

(3) There is facilitation of predicted release when one stimulus follows another by a short delay. The decay of this facilitation, assayed in a series of two-stimulus simulations, shows a non-exponential decline, nevertheless it can be fitted to a sum of exponentials, with time constants of about 50 and 300 ms.

(4) There is a lag in the onset of transmitter release which is
  (a) not very sensitive to the shape of the calcium influx function, and
  (b) is not sufficient to account for all the delay in release at the frog neuromuscular junction.

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134.2 ELECTRICAL ACTIVITY AT MAMMALIAN MOTOR ENDINGS. A. Mallart and J. L. Brigant*. Lab. de Neurobiologie Cellulaire, C.N.R.S., 91190 Gif sur Yvette, France.

Recent reports have shown distinct Na and K channel distribution in mammalian myelinated nerves : Na channels being restricted to the nodes of Ranvier and K channels to internodes. We investigated ionic channel distribution in mammalian motor endings by recording membrane currents by means of external electrodes. Precise electrode positioning on presynaptic terminals was achieved by using the flat and thin triangularis sterni muscle of the mouse and interferential phase contrast (Nomarski) optics at x 500 magnification. Two component negative waveforms, preceded by a small positivity, were recorded only from an area close to myelin end, while two-component positive signals were obtained from the major part of terminal branches. This indicates inward current at proximal and outward current at distal portions of the endings. We used specific channel blockers to identify the nature of membrane currents. Second component of both distal and proximal signals was suppressed by bath application of TEA or 3-4 DAP but only the distal one was sensitive to local ionophoretic application of either drug. Ionophoretic TTX application to proximal portion suppressed locally the first component of inward current and depressed distal outward currents. In contrast, TTX application to distal portions failed to affect waveform configuration. These results indicate the presence of Na but no K channels close to myelin end and of K channels but no Na channels at terminal portions. The latter are, thus, expected to be passively depolarized by electrotonic spread from the initial portion and upstream nodes of Ranvier. Furthermore, inward Na current and outward K currents promote separate local circuits between proximal and distal portions which are responsible for early distal outward and late proximal inward passive currents respectively.

Complete suppression of K current revealed slow inward current at distal portions which could be identified as Ca current by its dependence on external Ca^{2+} and its sensitivity to Co^{2+}. Ba^{2+} and Sr^{2+} could substitute for Ca^{2+} as current carriers.

134.3 ABSENCE OF ^{125}I-α-BUNGAROTOXIN BINDING TO VERTEBRATE MOTOR NERVE TERMINALS. Stephen W. Jones and Miriam M. Salpeter. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The existence of nicotinic acetylcholine receptors (AChRs) on motor nerve terminals (NTs) has been particularly controversial ever since the first pharmacological evidence appeared (Mascand & Wigton, J. Neurophysiol., 3:269, 1940). This issue has recently been revived by the finding that horseradish peroxidase (HRP) conjugated to α-bungarotoxin (BGT) labeled NTs at the frog neuromuscular junction (NMJ), even after "disjunction" (separation of NTs from the muscle) with collagenase and protease (Lentz et al., Brain Res., 132:423, 1977). However, using electron microscope autoradiography (EM-AR) with ^{125}I-BGT, Matthews-Bellinger and Salpeter (J. Physiol. 279:197, 1978) found frog NTs to have 0-5% of the postsynaptic AChR site density. To determine the presynaptic AChR concentration more precisely we have used EM-AR to localize BGT binding at NMJs after "disjunction" to improve resolution. We previously reported that frog motor NT membrane has fewer than 20 sites/μm^2 following disjunction (Jones & Salpeter, Fed. Proc., 40:261, 1981), i.e., ~0.1% of postjunctional values. We report here that NT membrane of the frog, lizard, and mouse does not bind significant amounts of BGT. After correction for an elevated background in the area of the disjoined endplate, NTs in frog cutaneous pectoris muscle had 3±2 BGT binding sites per μm^2, mouse flexor digitorum brevis NTs had 6±10 sites/μm^2, and lizard intercostals had 3±10 sites/μm^2. The enzymatic digestion produced no significant change in the postsynaptic BGT binding on dense postsynaptic membrane. We conclude that there are essentially no presynaptic nicotinic receptors at the NMJs of three diverse vertebrate species. The histochemical results to the contrary may reflect binding to non-receptor sites. Explanation of the pharmacological results may involve muscarinic receptors (Michaelson et al., PNAS, 71: 1376, 1976) or effects such as potassium release from muscle (Hohlfeld et al., Pflugers Arch., 391:213, 1981).

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134.4 PHYSOSTIGMINE AND d-TUBOCURARINE HAVE PRESYNAPTIC ACTIONS AT THE NEUROMUSCULAR JUNCTION. G.G. Bierkamper* and W.R. Millington, (SPON: A. Pestronk). Laboratory of Neuromuscular Toxicology, Dept. of Environmental Health Sciences, The Johns Hopkins Univ., Baltimore, Maryland 21205.

Physostigmine (PS) is a reversible acetylcholinesterase (AChE) inhibitor which also appears to alter the release of acetylcholine (ACh) from the neuromuscular junction (NMJ) by a presynaptic action. d-Tubocurarine (d-TC), a competitive blocker of nicotinic ACh receptors, has also been shown to exert presynaptic actions at the NMJ, but without a clear understanding of whether it increases or decreases ACh release. We have investigated the effects of PS and d-TC on the release of ACh from a vascular perfused rat phrenic nerve-hemidiaphragm preparation (Bierkamper & Goldberg, J. Electrophysiol. Tech. 6:40,1978). Briefly, the left hemidiaphragm is cannulated via the inferior phrenic vein and perfused with HEPES-buffered medium containing glucose, salts, and choline. DFP (10^{-5}M), an anti-AChE agent, was present in the perfusion medium for the first 30 min of the PS experiments and for the entire course of the d-TC experiments in order to recover released ACh. ACh release was measured by intracellular recording techniques and by radioenzymatic assay of perfusate extractions. PS (7×10^{-6}M) significantly increased the amount of ACh released during 7 Hz stimulation as compared to DFP-treatment alone (12.75±0.91 pm/min/hemidiaphragm, mean±SEM, n=8 vs. 6.75±0.07, n=5). Unstimulated release was unaffected. In contrast to the enhanced release of ACh as measured biochemically, intracellular recordings at the NMJ indicated that PS (10^{-5}M) decreases mepp amplitude and frequency by 37% and 33%, respectively. Endplate potentials (EPP;7Hz) amplitudes were also decreased, but without a significant change in quantal content (m; 3.58±0.43 vs. 2.70±0.26; calculations based on a single cell which was impaled and held during an entire experiment; n=4). These results suggest that PS may have an antagonistic action on post-junctional ACh receptors and, perhaps, antagonizes a similar functional site presynaptically. ACh release in the presence of d-TC (10^{-4}—10^{-6}M) decreased significantly during 7Hz stimulation (from a control level of 9.0±0.8 to 4.1±0.7 pm/min after 70 min of continuous stimulation; d-TC 10^{-4}M). EPP amplitudes and m were also decreased. These results are discussed in terms of presynaptic mechanisms which may regulate the release of ACh at the NMJ.

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134.5 CADMIUM REDUCES RATE OF SPONTANEOUS TRANSMITTER RELEASE BY BLOCKING CALCIUM CHANNELS. R.S. Manalis and G.P. Cooper. Dept. of Environmental Health, Univ. of Cincinnati, Coll. of Med., Cincinnati, OH 45267.

Experiments were performed in order to determine if the reduction in the rate of spontaneous transmitter release brought about by cadmium ions (Cd^{++}) is the result of Cd^{++} interfering with the entrance of Ca^{++} into the nerve terminal or with another aspect of transmitter release (e.g., exocytosis). Experiments were performed on the frog (Rana pipiens) sciatic nerve-sartorius muscle preparation. The rate of spontaneous transmitter release was taken as the frequency of miniature endplate potentials (MEPP frequency), and evoked release was determined by the average amplitude (in mV) of the endplate potential (EPP). MEPP frequency was increased by applying short trains of tetanic stimuli (100 ips for 50 msec) every second in preparations bathed in 12 mM Mg^{++}/0.46 mM Ca^{++} - Ringer. The addition of 20-30 μM Cd^{++} to the Ringer resulted in a clear reduction of the MEPP frequency and a reduction of the EPP to zero. The MEPP frequency never fell to zero; in fact, after reaching a quasi steady-state, it began to increase even though Cd^{++} was still present in the bath. This apparent recovery was not observed for the EPP; the application of tetanic stimuli to the nerve was maintained throughout the entire experiment. In one experiment, however, nerve stimulation was discontinued after the MEPP frequency was reduced by Cd^{++}, and, in this case, no increase in MEPP frequency was subsequently observed. Therefore, the apparent recovery seen in the other experiments can be attributed to the maintained nerve stimulation. The reduction of the rate of spontaneous transmitter release by Cd^{++} is only observed when a significant proportion of the spontaneous release is due to the influx of Ca^{++}. Intracellular Ca^{++} and, thus, MEPP frequency, can be elevated by disrupting the Ca^{++} buffering mechanisms occurring within the nerve terminal. MEPP frequency was measured in preparations bathed in a modified Ringer containing 0 mM Ca^{++}, 6 mM Mg^{++}, and 1.5 mM sodium warfarin. MEPP frequency increased by more than 10x within 45-60 min following the addition of sodium warfarin. Such increases in MEPP frequency were not reduced by Cd^{++}. In other experiments, performed in the presence of Ca^{++}, the lead-induced increase in MEPP frequency was also not reduced by Cd^{++}. However, this action of lead was absent when a preparation was exposed to a modified Ringer containing both Cd^{++} and Pb^{++}. Presumably, Pb^{++} enters the nerve terminal via the Ca^{++} channels. It is concluded that Cd^{++}, as compared to other heavy metal ions, is a very specific Ca^{++} channel blocker. (Supported by US PHS NIH grants ES-00159 and NS-17968.)

134.6 A TOXIC PEPTIDE FROM THE VENOM OF A MARINE SNAIL BLOCKS CALCIUM EVOKED-RELEASE OF TRANSMITTER. Lynne M. Kerr & Doju Yoshikami. Dept. of Biology, University of Utah, Salt Lake City, Utah 94112.

A highly basic toxic peptide (~20 amino acids) has been isolated from the venom of the marine snail, Conus geographus, by the laboratories of Drs. B.M. Olivera and W.R. Gray in our Department. Within minutes after administration of this peptide (50nM-5μM) to the neuromuscular junction of the frog, there is a total and irreversible block of epp's following a transient increase in mepp frequency. After blockade, the muscle still responds to direct electrical stimulation and carbachol application. In addition, amplitudes of spontaneous mepp's appear unaltered. These results indicate that the peptide acts presynaptically. However, an increase in mepp frequency can still be induced by procedures that do not depend on entry of external Ca^{++} into the nerve terminal, i.e. by exposing the preparation to high $[K^+]_o$ or hypertonic solutions containing 2 mM EGTA. In view of the fact that transmitter release can still be evoked by these procedures following blockade of epp's, transmitter depletion is probably not involved in toxin action.

When the preparation is treated only briefly with the peptide, a permanent, partial reduction of the epp is obtained and observed to be due to a decrease in quantal content. This condition is maintained over many hours, suggesting that the nerve has not been damaged non-specifically by the toxin. The reduction in quantal content can be partially antagonized by elevating $[Ca^{++}]_o$. Thus, the peptide produces a state resembling that normally achieved by lowering $[Ca^{++}]_o$. The results thus far are consistent with the hypothesis that the peptide acts by blocking Ca^{++} entry during the presynaptic action potential. Further experiments are underway to distinquish between this hypothesis and other possibilities.

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134.7 CALMODULIN INTERACTION WITH PURIFIED SYNAPTIC VESICLES FROM NARCINE ELECTRIC ORGAN. J.E. Hooper* and R.B. Kelly, Division of Neurobiology, Univ Cal School of Medicine, San Francisco, CA 94143

Synaptic vesicles participate in at least two important calcium-mediated functions in the nerve terminal calcium (uptake/sequestration) and exocytosis. Calmodulin which is present in substantial amounts in nerve terminals of the Narcine electric organ may have an important role in regulation of synaptic vesicle functions. Recently Schweitzer and Kelly (Neurosci. Abst. 1982) have shown that trifluperazine which blocks calmodulin responses blocks depolarization-dependent release of ATP from marine electric organ synaptosomes (I_{50}= 3uM) without affecting $^{45}Ca^{++}$ uptake.

Using ^{125}I labeled calmodulin as a probe we find that intact synaptic vesicles purified from Narcine electric organ show saturable calmodulin binding (K_D~15nM). This binding is calcium dependent (apparent K_D~10uM) and inhibited by trifluperazine (I_{50}~8uM). Non equilibrium kinetic analysis reveals a major fast (~70%) and minor slow (80%) component for both on and off rates. The respective constants for the fast and slow components are k_{on}=>$8x10^6$ $M^{-1}sec^{-1}$, k_{off}=>$1.2x10^{-2}sec^{-1}$ and k_{on}=$1.4x10^4$ $M^{-1}sec^{-1}$; k_{off}=>$2.9x10^{-4}sec^{-1}$. While stoichiometric vary in different preparations, values corresponding to up to 6 calmodulin binding sites/synaptic vesicle have been observed. The binding activity is associated with the vesicles rather than contaminants: it copurifies with vesicle markers in the last step of the vesicle purification. Furthermore calmodulin covalently bound to Sepharose beads can efficiently precipitate intact synaptic vesicles. We conclude that synaptic vesicles have calmodulin binding sites on their cytoplasmic face. The physiological role(s) of these calmodulin binding sites remain to be elucidated.

134.8 4-AMINOPYRIDINE AUGMENTS ELECTRICAL AND CHEMICAL TRANSMISSION IN IN THE AVIAN CILIARY GANGLION. M.C. Homonoff* (SPON: A. Steinacker). The Rockefeller University, New York, New York 10021.

4-Aminopyridine (4AP) has been shown to increase stimulus evoked transmitter release at several synapses. This work reports that 4AP enhances both electrically and chemically-mediated transmission in the parasympathetic avian ciliary ganglion. 4AP was applied to the chick ciliary ganglion mounted on suction electrodes and transmission through the ciliary nerve was studied. Each ganglionic synapse supplying the ciliary nerve has an electrical and chemical component of transmission and the two components can be identified in extracellular recordings of the compound action potential as a first (electrical) and second (chemical) peak with a delay between them. Some of the electrically coupled junctions also transmit antidromically. After treatment of a ganglion with 0.1 mM 4AP in standard avian Tyrode's solution, with 5 mM Ca^{2+} and 2 mM Mg^{2+}, a single stimulus elicited a compound action potential with the first electrical peak increased in amplitude and prolonged in duration, the second chemical peak diminished in amplitude, and several new smaller peaks which occurred after delays of more than 5 msec. The amplitude and duration of the antidromic response were also increased by 4AP. Addition of 4AP to modified avian Tyrode's solution containing low Ca^{2+} and 6 mM Mg^{2+}, .05 mM d-tubocurarine, or 0.1 mM hexamethonium bromide, increased the amplitude and time course of a single electrical peak of the action potential, but additional small late components of the compound action potential were not observed. The data show how two modalities of transmission may interact at a single synapse. An interpretation of the results is that 4AP enhances the electrically-mediated component of transmission by increasing the duration of the presynaptic action potential; the later components of the compound action potential may be due to prolonged release of neurotransmitter. A second possibility is that antidromic stimulation through the electrical synapse following orthodromic stimulation of the postsynaptic cell may lead to further stimulation of release of acetylcholine. Intracellular pre- and postsynaptic impalements are planned to determine the mechanism for enhanced transmission. I would like to thank Dr. B. Ceccarelli of the Center for the Study of Peripheral Neuropathies in Milan, Italy for making available his facilities for research. This work was supported by PHS Grant NS05872.

134.9 TWO PRESYNAPTIC POTASSIUM CURRENTS AT THE SQUID GIANT SYNAPSE. G. Augustine and R. Eckert. Dept. of Biology and Ahmanson Laboratory of Neurobiology, UCLA, Los Angeles, CA 90024 and Winter Squid Program, Catalina Marine Science Center.

Potassium currents are partially responsible for repolarization of the action potential, and thus are presumed to influence transmitter release at synapses. Little, however, is known about the K currents of presynaptic terminals. To investigate presynaptic K currents, we voltage clamped the most distal giant preterminal located in the stellate ganglion of Loligo opalescens, using 2- and 3-microelectrode techniques. Holding potentials were -65 or -70 mV. Na currents were eliminated with bath application of $2x10^{-7}$g/ml TTX, and the temperature was 15°C.

Depolarizing steps elicited delayed outward currents that were separable into two components. One component, blocked by Cd^{2+} (0.2-5mM) or by lowering external Ca concentration, can be ascribed to a calcium-activated potassium conductance. The Ca-insensitive component resembles the voltage-gated delayed rectifier present in the squid giant axon. These two currents exhibit certain contrasting properties.

The delayed rectifier current activates fully within 20 ms, and shows no inactivation during depolarizing pulses as long as 300 ms. Maximum current density at 0 mV is approximately 1 mA/cm^2. Tail currents decay exponentially with a time constant of 3-5 ms at -65 mV, and reverse at approximately -80 mV. The delayed rectifier current is blocked by external 3-,4-diaminopyridine (DAP; K_D 10 μM), with the block removed in a use-dependent manner. It is blocked only slightly by 100 mM external tetraethylammonium (TEA).

The calcium-activated K current is much smaller, activates more slowly, and exhibits different pharmacological sensitivity. This current grows gradually during a 300-ms depolarization, without reaching a peak. Current density after 100 ms at 0 mV is approximately 100 $\mu A/cm^2$. Calcium-sensitive outward tail currents decay exponentially with a time constant of ~0.1 s at -65 mV. These tail currents also reverse at about -80 mV. The Ca-activated K current is selectively eliminated by external TEA (K_D 10mM), but is not reduced by DAP at concentrations up to 2mM.

In summary, squid presynaptic terminals produce at least two kinetically and pharmacologically distinct K currents. Both currents may influence presynaptic membrane potential and thereby synaptic transmission.

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134.10 STIMULATION AND DEPRESSION OF MAMMALIAN NEUROMUSCULAR TRANSMISSION BY METHYLMERCURY. William D. Atchison*, Allen W. Clark and Toshio Narahashi (SPON: C.H.Wu). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611 and Dept. of Anatomy, Univ. of Wisconsin Sch. of Med., Madison, WI 53706.

Our previous study has shown that bath application of methylmercury (20-100 μM) causes an irreversible block of neuromuscular transmission in the rat phrenic nerve-hemidiaphragm preparation (W.D. Atchison and T. Narahashi, The Toxicologist 2, 57, 1982). This block is characterized by an increase in the frequency of miniature end-plate potentials (MEPPs) after 20-45 min of exposure, and a gradual reduction to complete block of the evoked end-plate potential. The amplitude histograms of MEPPs are not significantly altered. With prolonged exposure (60-120 min), all synaptic activity ceases. The present study had three goals. The first, was to determine whether the end-plate sensitivity to acetylcholine (ACh) was altered by methylmercury either at the time when evoked release was blocked, or when MEPPs could no longer be recorded. The second was to determine whether the increase in MEPP frequency was dependent upon extracellular Ca^{2+} concentration. The third was to determine whether ultrastructural damage to the motor nerve terminals could be detected following methylmercury exposure. Electrophysiological experiments were conducted using the phrenic nerve-hemidiaphragm of rats (male, 120-180 g) and conventional microelectrode recording techniques. Ultrastructural studies were conducted on the diaphragm of mice (male, 20-25 g). At a concentration of 100 μM, methylmercury did not significantly depress the end-plate response to iontophoretic application of ACh at the time of end-plate potential block (5-15 min). Normal ACh-induced depolarizations could be elicited even after 1 hr treatment with methylmercury. The increase in MEPP frequency caused by methylmercury appeared to be independent of the external Ca^{2+} concentration. Over a range of bath Ca^{2+} concentrations (0.5, 2, 8 mM), increased MEPP frequencies of 4-8/sec were observed following 20-30 min exposure to 100 μM methylmercury. Control MEPP frequencies over the same range of Ca^{2+} concentrations were 0.5-1.3/sec. Ultrastructural studies of mouse diaphragms perfused for up to 1 hr with 40 μM methylmercury detected no evidence of altered fine structure. The number and morphology of synaptic vesicles and nerve terminal mitochondria were normal. The results of the present study suggest that methylmercury does not depress the sensitivity of the end-plate membrane to ACh and that the effects of methylmercury to increase spontaneous transmitter release are not mediated by the calcium-dependent release process. Supported by NIH grants ES05207, ES02330, and NS11445.

135.1 A STUDY OF THE NICOTINIC ACETYLCHOLINE RECEPTOR USING A PHOTOISOMERIZABLE COMPETITIVE ANTAGONIST. M. E. Krouse*, H. A. Lester, B. F. Erlanger*†, and N. H. Wassermann*† (SPON: M. G. Price). Dept. of Biology, California Institute of Technology, Pasadena, CA 91125. †Dept. of Microbiology, Columbia University, College of Physicians and Surgeons, New York, NY 10027.

Competitive antagonists bind to unoccupied receptors, preventing agonist molecules from binding to the receptor and opening the receptor channel. At equilibrium, the effect of increasing antagonist concentration is to increase the number of closed receptor channels, as though the agonist concentration were reduced. In previous studies on the kinetics of receptor inhibition by antagonists such as d-tubocurarine, interpretations have been complicated by desensitization and by the high rates of action. We have developed a more appropriate method for measuring the kinetics of agonist-receptor interactions.

The photoisomerizable azobenzene derivative, cis-2, 2'-bis[α-(trimethyl-ammonium)methyl] azobenzene (2BQ), has been studied with the voltage-clamped Electrophorus electroplaque preparation. Dose-response studies were conducted in the presence of an agonist (carbachol) and 2BQ over a voltage range of -50 mV to -150 mV. At cis-2BQ concentrations less than 5 μM, the dose-response curves are shifted in a parallel fashion to higher agonist concentrations. A dose-ratio analysis reveals that cis-2BQ is a pure competitive antagonist with a voltage-independent dissociation constant of 0.5 μM, roughly equal to that of d-tubocararine. Because 2BQ can exist in two stable photoisomers, it was also of interest to compare the effects of several 2BQ solutions with varying ratios of the cis/trans isomers. The apparent affinity of the receptor for 2BQ varies linearly with the mole fraction of the cis isomer; the extrapolated dissociation constant is >10 μM for the pure trans isomer.

These studies, on the equilibrium between receptors and 2BQ, provide a basis for several kinetic experiments employing light flashes:

1) Rapid changes of the cis-2BQ concentration;
2) Structural perturbations of the antagonist-receptor complex;
3) Studies of the possible effects of 2BQ on the open receptor channel.

135.2 SINGLE-CHANNEL CURRENTS FROM CHOLINERGIC RECEPTORS IN CULTURED MUSCLE. L.D. Chabala *, H.A. Lester, and R.E. Sheridan. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

We are studying single ionic channels in cultured muscle. Mechanically dissociated myoblasts (from 10-12 day in ovo chick pectoral muscles) were allowed to fuse on Sylgard- or collagen-coated petri dishes, forming myoballs or myotubes, respectively. Cells from the cloned mouse line, BC3H-1, were grown as a monolayer on collagen and experiments were performed 10-12 days after seeding (5-7 days after reaching confluence). Currents through ionic channels opened by nicotinic agonists were recorded using a patch electrode (3-7 megohms) filled with a cholinergic agonist. The application of gentle suction to the pipette resulted in a tight seal (20-80 gigohms) with the cell membrane, thus allowing currents to be resolved at the single-channel level. We have determined that the photoisomerizable compound trans-Bis-Q (an azobenzene derivative) is an effective agonist both in chick pectoral muscle and in the BC3H-1 cell line. This is interesting because, although trans-Bis-Q is a potent agonist for nicotinic acetylcholine (ACh) receptors in electric eels and related fish, macroscopic recordings have not revealed any potency as an agonist for ACh receptors in adult frog, mouse, or rat muscle. The cis isomer seems to evoke little or no current (either macroscopic or single-channel) from any of these preparations. We observed that channels opened by ACh (and by trans-Bis-Q) have at least two open conductance levels in embryonic chick muscle (see also Hamill & Sakmann, Nature **294**: 462, 1981). The principal conductance level is approximately 40 pS and the smaller conductance level is 1/4 to 1/3 as large. When these two conductance levels are observed, the lower conductance level accounts for 5-10% of the open times and is usually seen as a transition from the higher conductance level. The channel then either reopens to the principal conductance level or closes. A few isolated openings to the lower conductance level have been observed. The fact that trans-Bis-Q is an effective agonist in these preparations and that the cis/trans ratio can be modulated with light of a known wavelength suggests several light-flash experiments in which Bis-Q is photoisomerized while interacting with a receptor in a known state.

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135.3 THE EFFECTS OF THE NEUROMUSCULAR BLOCKING AGENT ORG.6368 ON THE ENDPLATE CHANNELS OF AMPHIBIAN MUSCLE. N. N. Durant, R. Horn* and J. J. Lambert*. Departments of Anesthesiology and Physiology, UCLA School of Medicine, Los Angeles, Ca 90024 and Department of Pharmacology, Univ. of Dundee, Dundee, Scotland.

Org.6368 is a steriodal bisquaternary nondepolarizing neuromuscular blocking agent with a chemical structure which is similar to pancuronium with both agents being based upon an androstane nucleus. The actions of Org.6368 (25 to 200 μM) were investigated at the endplate region of the isolated cutaneous pectoris muscle obtained from Rana pipiens. A conventional twin microelectrode voltage clamp technique was used to record the peak amplitude and decay rate of evoked endplate currents at holding potentials ranging from -140 to +50 mV. The recorded data was then subjected to analysis on the basis of the sequential kinetic model for drug-blockade of open acetylcholine-activated channels originally proposed by Steinbach (J. gen. Physiol. 52, 162: 1968);

$$S_0 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} S_2,$$

where S_0 is the inactive closed channel, S_1 is the acetylcholine-activated open channel and S_2 is the open channel blocked by Org.6368. The rate constant for the opening of the channels by acetylcholine released from the nerve terminal, k_1, was assumed to be very small; k_{-1} was determined from the exponential decay rate of endplate currents recorded from transected muscle in the absence of drug. The blocking rate constant, k_2, is a linear function of drug concentration. Analysis of the data indicates that k_2 is of the order of 10^6 M^{-1} s^{-1} at 0 mV and increases e-fold for a hyperpolarization of holding potential of about 140 mV. The unblocking rate constant, k_{-2}, also increases with hyperpolarization (approximately e-fold/40 mV) and has a value of the order of 30 s^{-1} at 0 mV. This type of voltage dependency of k_{-2} has not been reported with other neuromuscular blocking agents such as d-tubocurarine or gallamine nor with the local anesthetics. In the present study Org.6368 produced no change in the reversal potential and there was no evidence of deviation from linearity of the relationship between peak endplate current amplitude and holding potential.

The results of the present study suggest that the interaction between Org.6368 and the acetylcholine-activated ionic channel is more complex than the above model. One possible explanation is as follows; once the drug binds to the receptor-channel complex the channel undergoes a conformational change to a non-conductive drug-bound state. The return from this state is enhanced by hyperpolarization.

135.4 THE EFFECTS OF DIVALENT IONS ON CHANNEL BLOCKING AGENTS AT THE MOUSE NEUROMUSCULAR JUNCTION. James McLarnon* and D. M. J. Quastel. Dept. of Pharmacology, Faculty of Medicine, University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

Raised concentrations of Mg^{2+} or Ca^{2+} at the mouse neuromuscular junction modify the time course and height of miniature end-plate currents (MEPCs). Abbreviation of MEPC duration by Mg and lack of effect of Ca contrast with the effect of muscle fibre hyperpolarization to prolong MEPCs, suggesting that the divalent ions exert specific actions on the kinetics of closing of acetylcholine activated channels. To examine whether these ions have actions related to surface charge screening, we have determined the effects of raised Mg^{2+} and Ca^{2+} on the action of a number of channel blocking agents, including atropine, lidocaine, procaine, scopolamine and quinidine, which all act in a voltage-sensitive manner, and pentobarbital and menthol, which act independently of membrane potential. The rate constants for channel "plugging" and "unplugging" were estimated assuming a sequential model. The results show that the divalent ions act to decrease both the rate of channel blocking and the rate of channel unblocking for the positively charged agents, for which both rate constants are voltage-sensitive. The magnitude of the decrease did not vary appreciably between agents and was about 35% for the onward rate constant and 30% for the off rate constant, with 20 mM Ca^{2+} or Mg^{2+}. The rate constants were not significantly altered with raised divalent ion concentration for pentobarbital or menthol. These results suggest that the divalent ions alter the field strength in the membrane in the immediate vicinity of the activated receptor, thereby acting in the same way as hyperpolarization with respect to the rate of dissociation of positively charged drugs from their binding sites. At the same time, neutralization of negative surface charges in or near the entrance of the open channel reduces the rate at which such drugs can approach and bind to these sites.

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135.5 EFFECT OF HIBERNATION AND TEMPERATURE ON THE ACETYLCHOLINE RECEPTOR-IONIC CHANNEL COMPLEX IN 13-LINED GROUND SQUIRRELS. S.S. Deshpande*, A.C. Oliveira* and E.X. Albuquerque. Dept. Pharm. & Exp. Ther., U. of Md Sch. of Med, Baltimore, MD 21201.

Neuromuscular transmission in skeletal muscles of hibernating ground squirrels (C. tridecemlineatus) is maintained even at 5-7°C (Exp. Neurol., 62, 1978). An earlier communication (Fed. Proc., 37, 1978) has also shown that neither hibernation nor denervation (up to 30 days) during hibernation alters endplate currents (EPCs) of squirrel muscles. In continuation of these experiments, the effects of temperature on miniature EPC (MEPCs) and acetylcholine (ACh)-induced noise were studied in innervated and denervated soleus muscles of hibernating and innervated muscles of nonhibernating squirrels using voltage clamp and fluctuation analysis techniques. The rise time of MEPCs was shortened significantly when the temperature was increased from 10 to 30°C in innervated muscles of both nonhibernating and hibernating animals. In both groups the amplitude and decay time constant (τ) of MEPCs were voltage dependent and increased as the holding potential became more negative or as the temperature was increased from 10 to 30°C. At -100 mV the mean τ values of 12.68, 3.02 and 0.81 msec obtained at 10, 20 and 30°C, respectively, in soleus muscles of nonhibernating animals were not significantly different from those obtained at similar temperatures in the muscles of hibernating animals (10.79, 2.37 and 0.79 msec). The rise time and τ values for 25 day denervated soleus muscles of hibernating animals were comparable to those observed for the innervated muscles from the same group. The mean equilibrium potential of the MEPCs in innervated and denervated muscles of both groups at 20°C was about +3 mV. Fluctuation analysis of ACh induced noise showed mean (γ) of 23.4 and 21.9 pS at -60 and -80 mV, respectively at 10°C and 27.7 and 24.5 pS at 20°C in the innervated muscles of nonhibernating animals. The mean single channel lifetime (τ_I) at 10°C was significantly higher than that observed at 20°C (4.37 vs 1.12 msec). Similar temperature dependent increase in τ was also seen in the muscles of hibernating animals. These results clearly indicated that hibernation per se does not alter γ or channel τ_I in innervated or denervated muscles where neuromuscular transmission is present. An increase or decrease in temperature has profound effects on the risetime of τs of MEPCs and the shift in the values of these parameters is parallel in the muscles of both hibernating and nonhibernating animals. (Supported by USPHS grant NS12063-08.)

135.6 INTERACTIONS OF BUPIVACAINE WITH THE IONIC CHANNEL OF NICOTINIC RECEPTORS. S.R. Ikeda*, R.S. Aronstam* and E.X. Albuquerque (SPON: L. Goldman). Dept. Pharm. & Exp. Therap., U. Md Sch. Med, Balt., MD 21201 and Dept. Pharm., Med. Col. of GA, Augusta, GA 30912.

The effects of the tertiary local anesthetic bupivacaine HCl on nicotinic acetylcholine ionic channels were studied using electrophysiological and biochemical methods. Voltage clamp studies of the frog sartorius and cutaneous pectoris neuromuscular junction revealed a concentration-dependent depression of the time constant of endplate current (EPC) decay. Voltage dependence of EPC over the range +50 to -150 mV was reduced at all concentrations tested (25-100 µM). Under all conditions the EPC decay was adequately described by a single exponential. Peak EPC amplitude was also depressed in a concentration-dependent manner such that 100 µM bupivacaine reduced peak amplitude by 50%. The current-voltage relationship remained linear under all conditions tested. Nerve evoked responses were difficult to study at concentrations greater than 100 µM due to apparent blockade of nerve conduction. Extracellular recording showed results similar to those obtained with EPCs. These studies indicate that the time constant of EPC decay could be reduced to less than 250 µsec at drug concentrations of 400-800 µM. Preliminary studies of single ionic channel events using patch clamped rat myoballs are consistent with the above findings. Single channel lifetime was reduced by 50-100 µM bupivacaine with no evidence of "flickering". In addition, single channel conductance appears to be only slightly reduced (~ 20%).

Biochemical studies on Torpedo californica membrane fragments were performed using [^{3}H]phencylidine as a channel probe. In these studies, bupivacaine inhibited the binding of [^{3}H]phencylidine (3 nM) by 50% (IC_{50}) at 32 µM. If the membranes are preincubated with carbamylcholine, the IC_{50} is reduced to 6 µM. Interaction of bupivacaine with the (ACh) receptor site (agonist binding site) is minimal when determined by [^{3}H]ACh binding (IC_{50} greater than 1000 µM).

These results suggest that bupivacaine reacts primarily with the ionic channel of the nicotinic AChR while having minimal effects on the agonist binding site. Although the shortening of channel lifetime and biochemical studies are consistent with the present model for "open channel" block other schemes have not been excluded. (Supported by U.S. Army Contract DAMD-17-81-C-1279).

135.7 A CATIONIC MEMBRANE PROBE, TRIPHENYLMETHYLPHOSPHONIUM, ALTERS AND BLOCKS ACETYLCHOLINE RECEPTORS AT THE FROG NEUROMUSCULAR JUNCTION. C.E. Spivak*, M.A. Maleque* and E.X. Albuquerque. (SPON: A.F. BOYNE). ARC, NIDA, Balt., MD 21224 and Dept. of Pharm. & Exp. Ther., Univ. of MD. Sch. of Med., Balt., MD 21201

Triphenylmethylphosphonium (TPMP) is a hydrophobic cation with diffuse enough positive charge to partition into and traverse phospholipid membranes. Inasmuch as a wide variety of amphipathic or hydrophobic amines alter the acetylcholine receptor ion channel complex (AChR) of the neuromuscular junction, and that a number of authors cite the possibility that they do so via the membrane phase, we predicted that TPMP, too, should alter the AChR in typical ways.

The nerve-evoked twitch of frog sartorius muscles was blocked by TPMP at concentrations between 10 and 20 µM. This blockade readily reversed within 30 minutes by washing the muscle with Ringer's solution. The directly elicited twitch was unaffected.

Frog sartorius fibers were voltage clamped and endplate currents (EPC) were elicited. The slope conductance (at 0 mV) of the EPC peak amplitude was decreased about 50% in 20 µM TPMP. The current-voltage relationship showed upward concavity centered at around -100 mV. This concavity was shown to arise from time-voltage dependence. Thus, when EPCs were elicited 5 or 10 mS after a jump in membrane potential from -50 mV to other potentials (decade increments), the current-voltage relationship became linear. The time constant for voltage-time dependence was about 1.5 S in 20 µM TPMP and longer in 10 µM TPMP.

The time constant (τ) for EPC decay was shortened as the TPMP concentration increased. The semilogarithmic plot of τ vs. membrane potential was linear, but slope and the predicted τ at -80 mV both decreased as the TPMP concentration increased. The plot of $1/\tau$ vs. [TPMP] was linear, which is consistent with the open channel blockade mechanism. Patch clamp experiments on rat myoballs at 15°C confirm that channel lifetime is shortened and show further that average single channel conductance is also decreased (by about 15%) in the presence of 10 µm TPMP.

We conclude that TPMP is a potent blocker of the AChR. Its actions qualitatively resemble those of many other noncompetative blockers. Its hydrophobic character reinforces the importance of hydrophobic interactions at the AChR. (Supported by USPHS grant NS-12063.)

135.8 THE EFFECTS OF THE ANTIAMEBIC DRUGS EMETINE AND DEHYDROEMETINE ON THE ACETYLCHOLINE ACTIVATED RECEPTOR-IONIC CHANNEL. Karim A. Alkadhi* (SPON: M. Lokhandwala). Dept. of Pharmacology and Institute of Cardiovascular Studies, University of Houston, Houston, TX. 77004.

Emetine and dehydroemetine are known to cause muscle weakness when taken for the treatment of amebiasis. To investigate the mechanism of this side effect, the actions of these drugs were examined at the amphibian neuromuscular junction. Electrophysiological recording techniques showed that in the magnesium-blocked intact cutaneous pectoris muscle of the frog, both emetine(100 µM) and dehydroemetine (100 µM) decreased the endplate potentials (epps) as well as the miniature endplate potentials (mepps) with no apparent effect on the resting membrane potential. Voltage clamp studies in the transected muscle showed that emetine produced a concentration dependent reduction of both the time constant of decay (τ) and peak amplitude (Ip) of the stimulus-evoked endplate current at all holding potentials. At a holding potential of -90mV, emetine (100 µM) caused 31± 10% reduction of Ip and 64±5% (n=3) reduction in τ of the endplate current. These effects appeared within two minutes after infusion of the drug. During washout of emetine, Ip rapidly recovered to control values within 5 min. whereas τ was slower to recover. Normally, τ is voltage-dependent; being longer at hyperpolarized holding potentials. In the presence of emetine the normal relationship between τ and holding potentials was reversed and τ became longer at depolarized potentials. The normal single exponential nature of the decay of the endplate current was unchanged at all holding potentials. Although emetine depressed the Ip of the endplate current at every holding potential, the linearity of the current voltage relationship was not altered. There was also no change in the reversal potential. These findings suggest that emetine blocks the acetylcholine activated ion channels at the endplate which contributes to its reported myotoxic effect. (Supported by BRSG-College of Pharmacy and by a research grant from the Epilepsy Foundation of America).

135.9 QUINUCLIDINYL BENZILATE ACTIONS AT NICOTINIC CHOLINERGIC SYNAPSES: BIOCHEMICAL CHARACTERIZATION. James L. Graham*, Latha Narayanan* and Robert S. Aronstam (SPON: J.J. Buccafusco). Department of Pharmacology, Medical College of Georgia, Augusta, GA 30912.

3-Quinuclidinyl benzilate (QNB), a psychotomimetic glycolate ester with potent antimuscarinic activity, has multiple pre- and postsynaptic effects at the neuromuscular junction. In electrophysiological experiments, Schofield et al. (Cell. Molec. Pharm. 1:209, 1981) demonstrated QNB blockade of electrically-excitable sodium channels as well as receptor-associated ion channels in the open conformation. We have characterized the binding of QNB to several sites on the nicotinic receptor-ion channel complex in Torpedo californica electric organ using various radiolabelled probes, and have investigated the use of [^{3}H]QNB as a direct probe for extra-receptor binding sites which are sensitive to the conformational state of the receptor-channel complex.

QNB inhibited the specific binding of 2 nM [^{3}H]perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX) and [^{3}H]phencyclidine ([^{3}H]PCP) to sites associated with drug blockade of the synaptic ion conductance mechanism with IC50 values between 3 and 30 μM. Other muscarinic antagonists, including atropine and scopolamine were at least 100 fold less potent in this regard. Binding was measured using filtration procedures. QNB affinity for these sites was 3-4 fold greater in the presence of 1 μM carbamylcholine. QNB inhibition was slowly reversible: Only 60% of specific [^{3}H]PCP binding was recovered after three centrifugation washes of Torpedo membranes which had been exposed to 100 μM QNB for 20 min. QNB affinity for these sites was the same when measured at 0° or 30°. In contrast, QNB had little affinity for the ACh binding site, measured by equilibrium dialysis using [^{3}H]ACh or [^{3}H]d-tubocurarine, or by filtration using [^{125}I]alpha-bungarotoxin.

Radiolabelled QNB binding to Torpedo membranes was measured by equilibrium dailysis. The binding of 5 nM [^{3}H]QNB was doubled in the presence of 1 μM carbamylcholine. This binding was inhibited by a number of compounds which block channel conductance in biophysical experiments, although drug affinity for [^{3}H]QNB sites frequently differed from drug affinity for the [^{3}H]H_{12}-HTX or [^{3}H]PCP binding sites. Carbamylcholine (1 μM) generally potentiated ligand binding to the [^{3}H]QNB site. These findings support the notion that QNB blocks transmission at the neuromuscular junction through interaction with sites on the postsynaptic receptor-ion channel complex which are sensitive to the conformational state of the complex. (Supported by a grant from the National Institute on Drug Abuse, DA 02834.)

135.10 AMINOPYRIDINE INTERACTIONS WITH ACETYLCHOLINE RECEPTOR COMPLEXES AT NICOTINIC SYNAPSES. Robert S. Aronstam. Dept. Pharmacology, Medical College of Georgia, Augusta, GA 30912.

Aminopyridines enhance the release of neurotransmitters by increasing presynaptic calcium influx. Altered postsynaptic sensitivity has not been implicated in the actions of aminopyridines at the neuromuscular junction insofar as the amplitudes and time courses of miniature endplate potentials are not affected. The interactions of aminopyridines with ACh receptors and their associated ion channel binding sites were assessed in Torpedo californica electric organ using specific radiolabelled probes for a number of sites on the receptor-channel complex. The drugs tested were 2-, 3-, and 4-aminopyridine (2-AP, 3-AP, and 4-AP) and 2,3-, 3,4-, and 2,6-diaminopyridine (2,3-DAP, 3,4-DAP, and 2,6-DAP). 4-AP, 2,3-DAP and 3,4-DAP were approximately equipotent in inhibiting [^{3}H]ACh binding to the receptor measured by equilibrium dialysis. 2-AP and 3-AP were significantly less potent (IC50 = 10 mM), while 2,6-DAP did not inhibit [^{3}H]ACh binding at 10 mM. At low concentrations, several aminopyridines stimulated the binding of [^{3}H]phencyclidine ([^{3}H]PCP) and [^{3}H]perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX) to sites associated with drug blockade of the ion conductance mechanism. In terms of their ability to stimulate [^{3}H]PCP and [^{3}H]H_{12}-HTX binding, the following series was obtained: 4-AP > 2,3-DAP = 3,4-DAP > 2-AP = 3-AP (2,6-DAP did not stimulate channel binding). D-Tubocurarine moderated this channel binding stimulation. At higher concentrations all of the aminopyridines inhibited [^{3}H]PCP and [^{3}H]H_{12}-HTX binding with the following order of potency: 4-AP = 2,3-DAP = 3,4-DAP > 2,6-DAP > 2-AP = 3-AP. The ability of each of the aminopyridines to inhibit [^{3}H]PCP and [^{3}H]H_{12}-HTX binding was enhanced in the presence of 1 μM carbamylcholine.

These results indicate complex interactions of aminopyridines with postsynaptic structures at nicotinic synapses which are apparently masked in physiological experiments by the large increase in transmitter release. (Supported by a grant from the National Institute on Drug Abuse, DA 02834).

135.11 POSTSYNAPTIC NEUROMUSCULAR BLOCKAGE BY CONOTOXIN GI. O.B. McManus* and J.R. Musick (SPON: J.W. Woodbury). Dept. of Physiology, U. of Utah, Salt Lake City, UT 84108.

The marine gastropod Conus Geographus possesses a well developed venom apparatus that it uses to prey on fish and for defensive purposes. The crude venom contains a number of toxic peptides (Gray et al., J. Biol. Chem., 256: 4734-4740, 1981), and a mixture of two of these (GI and GII) isolated from the crude venom has been shown to block neuromuscular transmission at a postsynaptic site, probably by binding to the acetylcholine receptor (McManus et al., Neurosci. Letts., 24: 57-62, 1981). The effects of synthetic GI (supplied by J.E. Rivier of the Salk Institute) on neuromuscular transmission were determined in the present study. GI blocked nerve-evoked contractions of mouse diaphragm muscle at concentrations (0.2-0.4 μM) similar to those of the native GI-GII mixture. GI also blocked nerve-evoked contractions of the frog cutaneous pectorus muscle, but at higher concentrations (3-4 μM). GI reduced or blocked membrane depolarizations at frog endplates caused by ionophoretic application of acetylcholine (ACh). Blockage of the response to ACh is not due to changes in the input resistance of the muscle fibre or the resting membrane potential. GI was slightly less effective in blocking miniature endplate potentials (mepps) at the frog endplate than in blocking ionophoretic potentials. This differential block of ionophoretic potentials and mepps might be due to: (1) Restricted access of toxin to receptor sites activated during mepps. This explanation is supported by the observations that the differential block was more pronounced soon after the toxin was applied and was reduced by treating the preparation with hyaluronidase. (2) Block of open ionic channels by GI. This type of use-dependent block would be more pronounced during long ionophoretic potentials than shorter duration mepps. GI produced a slightly greater reduction of later pulses during trains of ionophoretic potentials suggesting a use-dependent block. (3) Mepp amplitude may be less sensitive to blockade of receptors by an antagonist than the amplitude of ionophoretic potentials (Pennefather and Quastel, J. Gen. Physiol., 78: 313-344, 1981). These results are consistent with the hypotheses that Conus toxin GI blocks acetylcholine receptors at the frog motor endplate.

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135.12 END-PLATE BLOCKING ACTION OF LOPHOTOXIN. Stephen M. Vogel*, William D. Atchison* and Toshio Narahashi (SPON: S.C. Cheng). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Lophotoxin (LTX), a neurotoxin isolated from the Pacific sea whip Lophogorgia rigida, causes a progressive, irreversible block of nerve-evoked muscle contraction without affecting contractions evoked by direct stimulation (P. Culver and R. Jacobs, Toxicon, in press). In the present study, the effects of LTX on neuromuscular transmission were assessed in the rat phrenic nerve-hemidiaphragm preparation using conventional microelectrode techniques and in the frog cutaneous pectoris preparation using two microelectrode voltage clamp techniques. Contractions of frog muscles were abolished using the formamide method. LTX in the concentration range of 2-20 μM depressed the spontaneous miniature end-plate potential (MEPP) amplitude and the end-plate potential (EPP) amplitude. MEPP amplitude histograms were markedly shifted in the direction of low values by LTX. These effects occurred following a latency of 25-40 min. With increasing concentrations of LTX, the latency to onset of end-plate block decreased. The depressant effect of LTX on the MEPP and EPP amplitude progressed to virtually a complete block irrespective of the LTX concentration. The end-plate block induced by LTX could not be reversed by washing with toxin-free solution for 1 hr. The resting membrane potential of skeletal muscle fibers was unaffected by LTX. In voltage-clamp experiments on single end-plates, LTX (15 μM) depressed the end-plate current (EPC) amplitude nearly uniformly at potentials between -60 and +60 mV. LTX appeared not to affect the reversal potential for EPC. The EPC was noticeably depressed at 30 min and was reduced to 40-50 percent of control at 60 min. In contrast, the EPC remained stable for 60 min in control experiments performed on separate muscles. In LTX, the EPC continued to decline during 60 and 90 min of exposure and at concentrations of 15, 30, and 50 μM. The block of the EPC caused by LTX was not reversible; the block actually progressed further during washout of the toxin. At 5 μM, LTX had no effect on the EPC after 56 min. In summary, LTX irreversibly blocks the EPC and EPP. The site of action of LTX is proposed to be the acetylcholine receptor-channel.

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135.13

WITHDRAWN

135.14 CHEMICAL MODULATION OF DOPAMINE RESPONSE IN NEUROBLASTOMA CELLS. Akinobu Tsunoo* and Toshio Narahashi (SPON: R.A. Berenberg). Dept. of Pharmacol., Northwestern Univ. Med. Sch., 303 E. Chicago Ave., Chicago, IL 60611.

One of the mechanisms underlying the chemical or hormonal modulation of synaptic transmission is a change in intracellular cyclic nucleotide level. Cultured neuroblastoma cells are an excellent material for such study, since both biochemical and electrophysiological experiments can be conducted under well-controlled conditions. We examined whether dopamine- and acetylcholine (ACh)-induced depolarizations of neuroblastoma cells were affected by agents expected to change the cyclic nucleotide levels. Membrane potential was recorded by an intracellular microelectrode from cultured mouse neuroblastoma N1E-115 cells. Dopamine and ACh were applied to the cell by iontophoresis. Other drugs were applied by bath perfusion.

Iontophoretic application of dopamine generated a transient depolarization of the membrane. ACh caused a fast and transient depolarization. This response was blocked by d-tubocurarine but not by atropine, indicating that it is nicotinic in nature. In some cells, the fast depolarization was followed by a hyperpolarizing response and/or a slow depolarizing response, both of which were blocked by atropine but not by d-tubocurarine. Therefore they are muscarinic in nature. Dibutyryl cyclic AMP(0.2-1 mM) reduced the amplitude of the dopamine-induced depolarization without change in the membrane potential, but did not affect the nicotinic ACh-induced depolarization markedly. 3-Butyl-1-methyl-xanthine (0.5-1 mM), a cyclic nucleotide phosphodiesterase inhibitor, also depressed the dopamine-induced response by 36±15 (mean ± S.E.M., n=5). Like dibutyryl cyclic AMP, this inhibitor did not cause a marked effect on the nicotinic ACh response. Morphine (5 nM-2 μM) inhibited the dopamine-induced response without change in the membrane potential. The decrease in amplitude of the dopamine-induced depolarization by 1 μM morphine was estimated to be 29±10 (mean ± S.E.M., n=6). However, no effect of morphine was observed in the nicotinic ACh response in the same cells. D-Ala^2, D-Leu^5 - enkephalin (2 μM) also depressed the dopamine-induced response. It is suggested that changes in intracellular cyclic nucleotide levels modulate the dopamine-induced response but not the nicotinic ACh response of neuroblastoma cells.

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135.15 VOLTAGE CLAMP ANALYSIS OF FAST EXCITATORY SYNAPTIC CURRENTS IN BULLFROG PARASYMPATHETIC GANGLION CELLS. Elizabeth A. Connor and Rodney L. Parsons. Department of Anatomy and Neurobiology, College of Medicine, University of Vermont, Burlington, VT 05405.

The results of previous studies have indicated that the basic characteristics of fast synaptic excitatory currents (EPSCs) are similar in mammalian and amphibian sympathetic ganglion cells (1,2,4). In contrast, the EPSC decay characteristics of EPSCs in rat parasympathetic cells are quite different (3); the decay consisting of two exponential components. Analysis of Ach-induced current fluctuations ("noise") in rat parasympathetic cells gives two Lorentzian components which correspond closely to the two decay components. The present study was undertaken to determine the basic EPSC characteristics for amphibian parasympathetic cells in order to test whether the previously noted differences were unique to rat autonomic ganglion cells or were characteristic of parasympathetic cells in general. Fast EPSCs were recorded from voltage-clamped parasympathetic ganglion cells in the atrial septum of the bullfrog, Rana catesbeiana, maintained in a HEPES-buffered solution at 21-24°C. A two microelectrode voltage clamp was used to hold membrane voltage between +30 to -100 mV. EPSCs were collected during preganglionic stimulation at 0.38 Hz. Agonist-induced current fluctuations were recorded during iontophoretic application of acetylcholine. In 20 cells voltage-clamped to -50 mV the fast EPSC rose to a peak value of -4.6 ± 1.6 nA (m ± SD) within a few ms and decayed exponentially; the time constant of decay being 8.1 ± 1.6 ms. In 4 other cells, the decay at -50 mV appeared to be better fit with two exponential components; τ_1 and τ_2 being 6.0 ± 1.2 ms and 16.8 ± 4.2 ms, respectively. The peak EPSC-voltage relationship appears linear in most cells with the reversal potential equal to -3.0 ± 7.7 mV (7 cells). The decay time constant for EPSCs with single exponential decays increased with hyperpolarization; the value of the coefficient of voltage dependence being -0.0047 ± 0.0015 mV^{-1} in 5 cells. In other cells voltage-clamped in the range of -50 to -70 mV the power spectrum of Ach-induced "noise" was adequately fitted as a single Lorentzian function. The present results suggest that the basic properties of the fast EPSC in amphibian parasympathetic cells are more similar to those reported for sympathetic ganglion cells than for rat parasympathetic cells. Supported by NIH grant NS 14552 and by the MDA.

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135.16 VOLTAGE CLAMP ANALYSIS OF SLOW MUSCARINIC EXCITATION IN SYMPATHETIC NEURONS OF BULLFROG. S. M. McCort*, J. W. Nash* and F. F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The slow excitatory postsynaptic potential (EPSP) in vertebrate sympathetic neurons results from the muscarinic action of acetylcholine. The ionic basis of the slow EPSP has been studied for several years and both increased and decreased conductances have been reported. We have investigated the conductance mechanisms involved in the muscarinic excitation of sympathetic neurons using the single electrode voltage clamp. Type 'B' neurons in the IXth or Xth paravertebral sympathetic ganglia in bullfrog were clamped at a holding potential between -40 and -50 mV. Muscarinic excitation was elicited by superfusion with a 1 mM concentration of the muscarinic agonist methylcholine (MCh) for 1 min in the presence of 70 uM d-tubocurarine. Hyperpolarizing and depolarizing voltage steps were applied before, during, and after the application of methylcholine. Two types of experimental paradigm were used: (1) voltage steps, 500 msec in duration, from holding potential to membrane potentials of -25 mV, -35 mV, -70 mV, -90 mV and -110 mV; and (2) a sequential series of voltage steps, 500 msec in duration, as follows: -25 mV to -35 mV; -35 mV to -50 mV; -50 mV to -70 mV; -70 mV to -90 mV; and -90 mV to -110 mV. Current voltage curves constructed from data obtained by both methods were similar. In normal Ringer's solution, at depolarized membrane potentials the current was outward and at hyperpolarized membrane potentials the current was inward; the reversal between outward and inward current was usually at a membrane potential between -40 mV and -60 mV. At holding potentials of -40 to -50 mV, the application of MCh induced a slow inward current of 1-2 nA that reached peak amplitude in 1-2 min and gradually decayed over a period of 20-25 min. At the peak of the MCh response, the outward current at -25 mV and -35 mV was usually decreased and the inward current at -70 mV, -90 mV and -110 mV was increased. The reversal between outward and inward current at the peak of the MCh response was usually between -30 mV and -50 mV. Removal of both Na^+ (sucrose substitution) and Ca^{2+} (Mg^{2+} substitution) decreased or abolished the increased inward current induced by MCh at membrane potentials negative to -70 mV, but usually had relatively little effect on the current change induced by MCh between -35 mV and -70 mV. The results are consistent with at least two conductance changes in the generation of the muscarinic response: (1) a voltage-dependent decrease in K^+ conductance at membrane potentials between -35 and -70 mV (see Brown & Adams, Nature 283: 673, 1980; Weight & MacDermott, Physiol. & Pharm. Epileptogenic Phenomena, ed Klee et al, Raven, 1982, pp 227-233); and (2) a voltage-dependent increase in Na^+ and/or Ca^{2+} conductance at membrane potentials negative to -70 mV.

135.17 RELATIONSHIP OF S-EPSP AND ASYNCHRONOUS DISCHARGE IN MAMMALIAN SYMPATHETIC GANGLION. M. Crawford* and John H. Ashe. Department of Psychology, University of California, Riverside, California, 92521.

Repetitive stimulation of preganglionic nerve during nicotinic blockade by d-tubocurarine (35uM) elicits slow postsynaptic potentials (S-EPSP and S-IPSP) recorded from rabbit superior cervical ganglion (SCG) and asynchronous discharge (AD) from the postganglionic nerve, all of which are eliminated by the muscarinic antagonists atropine (1uM) or quinuclinidyl benzilate (0.05uM). These muscarinic responses have received considerable attention particularly with regard to the modulatory action of catecholamines (Ashe and Libet, Brain Res. 217: 93-106, 1981; McIsaac, JPET 207: 72-82, 1978). However, the functional relationship of these responses, which share similar pharmacological characteristics, has not been fully elaborated. In the present study the slow potentials and AD in response to orthodromic input to rabbit SCG were simultaneously recorded in the air-gap chamber and their relationship characterized with the aid of a computer.

When stimulus parameters are above the minimal necessary to elicit both responses, supramaximal stimulation at progressively higher frequencies and longer durations consistently produce parallel changes in S-EPSP and AD. Both responses increase in magnitude and duration. Stimulus parameters that were subthreshold (e.g., 20/sec. for 1 sec.) for AD did, however, produce S-EPSPs with amplitudes that could be as great as 80% of S-EPSPs produced by stimulus parameters optimal for AD.

In the presence of the cholinesterase inhibitor, eserine (1uM) or the specific acetylcholinesterase inhibitor, BW-284 (1uM), there were pronounced increases in the amplitude and duration of the S-IPSP. The more pronounced effect on the S-EPSP was an increase in duration without any apparent increase in amplitude; the AD increased in firing rate as well as response duration.

Discharge frequency appears to be governed by the rate at which the S-EPSP attains peak amplitude. During stimulus trains, the slope of the rising S-EPSP is reduced and full amplitude is not attained. This is apparently the consequence of decreased membrane resistance underlying the superimposed F-EPSP and the hyperpolarizing effect of the overlapping S-IPSP. Discharge usually begins at a low rate during the stimulus train and progressively increases with the slow rise of the S-EPSP. Following stimulus offset, the ascending slope increases and the S-EPSP rapidly attains its peak amplitude, accompanied by a pronounced increase in the rate of AD. Metoclopramide (100uM) decreases the rise time but also produces a pronounced enhancement of the S-EPSP (Ashe and Libet, in press). This slow rising but substantially enhanced S-EPSP is accompanied by considerably less discharge during the stimulus train and the usual pronounced discharge that follows the train is almost completely absent. (Supported by NIH BRSG RR07010-16)

135.18 SYNAPTIC TRANSMISSION IN THE INFERIOR MESENTERIC GANGLION OF THE RABBIT. M. A. Simmons and N. J. Dun. Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153.

The electrophysiological and pharmacological characteristics of synaptic transmission in isolated inferior mesenteric ganglia (IMG) of rabbits were investigated by means of intracellular recording techniques. The ascending, colonic, hypogastric and/or splanchnic nerves were drawn into separate suction electrodes for electrical stimulation. The passive and active electrical membrane properties of these cells were found to be comparable to those of other sympathetic neurons. With respect to synaptic transmission, stimulation of any one of the nerves elicited one to several f-epsp suggesting a marked convergence of synaptic inputs onto the principal ganglionic neurons. The f-epsp could be reversibly depressed by nicotinic antagonists; thus, it is likely to be generated by a nicotinic action of acetylcholine (ACh). Slow membrane potential changes were generally not observed following single presynaptic stimulation whereas they could frequently be elicited following repetitive stimulation (10-30 Hz, 1-5 sec). According to the electrophysiological and pharmacological characteristics of these slow potentials the IMG neurons could be classified into 4 groups. In the first type the f-epsp was followed immediately by a slow depolarization associated with an increased membrane resistance. As this depolarization was abolished by atropine (1 μ M), it appears to be mediated by a muscarinic action of ACh. In the second type the f-epsp was followed by a longer lasting depolarization which was not affected by atropine and thus is termed noncholinergic. This potential was always associated with an increased membrane resistance, usually about 30%, and was frequently accompanied by repetitive neuronal discharge. The mean amplitude and duration of this response was about 5 mV and 4 min, respectively. The third type of neuron appears to involve a combination of the above two responses with the initial portion being antagonized by atropine and followed by a noncholinergic component. In the final type of cell the slow depolarization was followed by a long lasting hyperpolarization that was not blocked by cholinergic or adrenergic antagonists. The f-epsp as well as all of these slow potentials were reversibly abolished in a low Ca/high Mg solution.

Our study clearly shows that synaptic transmission in the rabbit IMG is complex and may involve a number of transmitters in addition to ACh. The demonstration of the presence of a number of peptides in this ganglion by our immunohistofluorescence studies provides the basis for future study of the involvement of these peptides in synaptic transmission in the rabbit IMG. (Supported in part by NIH NS15848).

135.19 FURTHER EVIDENCE FOR A SUBSTANCE P MEDIATED EXCITATORY TRANSMISSION IN GUINEA PIG INFERIOR MESENTERIC GANGLIA. M. Kiraly*, Z. G.Jiang* and N. J. Dun. Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153.

Substance P (SP) has been implicated as the putative transmitter mediating the non-cholinergic excitatory potential which can be elicited by presynaptic stimulation in the majority of cells of guinea pig inferior mesenteric ganglia (IMG). We examined the effects of a SP antagonist, (D-Pro^2, D-Phe^7, D-Trp^9)-SP, and capsaicin, a compound known to cause a release and depletion of SP, on the non-cholinergic transmission to characterize further the nature of the transmitter in question. Intracellular recordings were obtained from neurons of the isolated IMG in vitro. Non-cholinergic depolarization was evoked by repetitive stimulation (20-30 Hz, 2 sec) of hypogastric nerves or ascending mesenteric nerve. SP analogue (1-50 μ M) reversibly depressed the non-cholinergic depolarization in a conc. dependent manner without affecting significantly amplitude of the f-epsp, resting membrane potential and input resistance of the majority of cells tested. In a few neurons SP analogue (> 10 μ M) caused a slow depolarization associated with an increase of membrane resistance. The membrane depolarization induced by bath application of SP (1 μ M) was antagonized by prior treatment of SP analogue. The effect of capsaicin on IMG neurons was distinctly different from that of SP analogue. Capsaicin (1-100 μ M) caused a slow and sustained membrane depolarization lasting for minutes. Capsaicin induced depolarization occurred only in those neurons that exhibited a non-cholinergic depolarization; whereas, capsaicin produced no detectable change of the resting membrane potential, input resistance and amplitude of the f-epsp in those neurons which did not show the non-cholinergic depolarization. During and after capsaicin-induced depolarization the non-cholinergic response elicited synaptically was attenuated and eventually abolished. The capsaicin induced blockade of non-cholinergic depolarization was nearly irreversible even after hours of wash with Krebs solution; small recovery (10-20 %) of the non-cholinergic depolarization could be observed in a few neurons after prolonged wash. Membrane depolarization induced by capsaicin was not antagonized by pretreating the ganglion with cholinergic nicotinic and muscarinic antagonists; it was prevented in a low Ca solution. In summary, the present findings that the non-cholinergic depolarization was specifically and reversibly depressed by a SP antagonist, and that capsaicin caused a depolarization and blockade of the non-cholinergic depolarization only in those neurons which exhibited the latter provide further pharmacological evidence that the transmitter responsible for the generation of the non-cholinergic depolarization is SP or a related peptide. (Supported in part by NIH NS15848).